

Manuscript Details

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Title	Properties of Partially Denatured Whey Protein Products: Viscoelastic Properties
Article type	Research paper

Abstract

Partially denatured whey protein products (PDWPC's) can be classified based on the viscoelastic properties of their solutions. Strain sweeps show that PDWPC-A and -B and microparticulated WPC (MPWPC) with compact, spherical aggregated particles exhibit a strong strain overshoot. PDWPC-C and -D, on the other hand, which have open, elongated porous particles show a weak strain overshoot. The concentration dependence of the elastic modulus G' in the linear viscoelastic region has a biphasic power law dependence with concentration for all protein products studied, except for WPC where G' is independent of protein concentration. Frequency sweeps suggest that MPWPC solutions form a strong physical gel at all concentrations above 14% (w/w). PDWPC-A and -B form weak gels over the same concentration range. PDWPC-C and -D also form weak gels at 14% protein (w/w) but strong physical gels at higher concentrations. The frequency dependence of G' and G'' for all aggregated proteins show a power law dependence indicating fractal type structures. For all solutions above a critical concentration, the fractal dimensions span the range 1.6-2.3, indicating a range of gel network structures from open and diffuse to compact and dense. Adherence to the empirical Cox-Merz rule was observed in PDWPC-A, -C and -D at concentrations of 14 and 16% (w/w) protein, suggesting liquid-like behaviour. At higher protein concentrations the deviations from the Cox-Merz rule suggest more pronounced elasticity in the structure. For PDWPC-B, the behaviour is complex, with deviation from the Cox-Merz rule at low frequencies/shear rates, but correspondence at higher frequencies/shear rates at all concentrations. This indicates a frequency-dependent change from liquid-like behaviour over long timescale deformations, to a solid-like behaviour at short timescale deformations. MPWPC solutions of all concentrations do not follow the Cox-Merz rule, suggesting solid-like behaviour. The PDWPCs exhibit a complex rheological behaviour which suggests they could be versatile thickening, texturizing and fat replacement ingredients that will offer food manufacturers greater control over the texture of formulated fluid foods.

Keywords	Partially denatured whey proteins; oscillatory rheology; frequency sweep; fractal; Cox-Merz rule
Taxonomy	Rheology of Dispersed System, Proteins, Novel Food Ingredients
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Order of Authors	Zhuo Zhang, Valeria Arrighi, Lydia Campbell, Julien Lonchamp, Stephen Euston
Suggested reviewers	John Coupland, Bettina Wolf, Anwesha Sarkar, Costas Nikiforidis

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The Editor
Food Hydrocolloids

10/10/17

Dear Sir/Madam,

Please find attached a revised manuscript entitled "Properties of Partially Denatured Whey Protein Products: Viscoelastic Properties". The manuscript present results for the frequency-dependent and concentration-dependent viscoelastic properties of solutions of partially denatured aggregated whey protein food ingredients that are produced using a novel technology allowing control over aggregation and particle properties.

Yours Sincerely,

Professor Stephen Euston

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Reviewer 1

This is a very well presented piece of research building on previously published work. I have only a few very minor comments:

(1) Abstract: Insert "a" between "exhibit strong" and also "show elastic"

Changed in text page 2 lines 25 and 27

(2) Abstract: "except for WPC where" you probably mean "MWPC"?

No this is correct. If you refer to figure 2 the WPC plot best fit line is almost horizontal whereas MPWPC is biphasic with two regions of differing but positive slope for the best fit lines.

(3) What is the pH of the solutions, could you add this information? A sentence has been added (Page 6, lines 106/107) "The pH of all solutions was in the range 6.7-7.0 and the pH was not adjusted before testing."

(4) Use G'/G" (apostrophe) instead of inverted comma

Altered throughout text.

Reviewer 2

General comments:

The paper is interesting and all the results seem to be reliable; however, the different techniques that authors performed are not enough for a complete comprehension of the study. Mainly because in the manuscript authors did not mention how the different products are obtained, which is the degree of denaturation of the products, which is the zeta potential of all the products in the case of the last study, etc. Even though these products are commercial and the authors have published other papers that can describe some of these characteristics, they must support these findings with more evidence or recalling previous results other than the different structures of aggregates (this is mentioned several times in the manuscript, and it is the only characteristic that authors mentioned).

On lines 108-112 brief details of the processing a degree of denaturation are included.

Specific comments

(4). The abstract is not clear and some important information is not mentioned such as how the products were obtained, which analysis were performed, which was the aim of the work, which means MPWPC solutions, etc

We disagree with the referee on this point. The abstract is to summarise the main finding of the work. If we were to include the details the referee requests we would exceed the maximum word count. The definition of MPWPC (microparticulated WPC) was given several times in the original manuscript (line 23, line 95/96).

(5). The manuscript does not contain the number of each lane. I strongly suggest to authors the use this tool in each manuscript submitted because it helps to revise the manuscript, and also to the authors when they have to find the suggestions, questions or queries of the reviewers.

Line numbers have been added

Figures

(6). The scale of the x-axis in all figures do not contain the minor scale, please add them.

Added in manuscript

(7) In the Figure 1 x-axis is not the same for all the figures, why? The y-axis in Figure 1 have different scales. I consider that in order to a better comparison all the y-axis should have the same scale.

We disagree with the reviewer on this comment. If we change the y-axis to 400 Pa, the maximum G' value in the lower of the graphs on Figure 1, it is not at all easy to see the features we refer to (ie weak and strong strain overshoot) in these figures. For clarity we have left the x-axes as they were in the original graphs.

Results

(8) In this sentence the words have been are twice: "These have been observed by others (Matsumoto & Inoue, 1996; Ikeda & Nishinari, 2000; Ikeda & Nishinari, 2001a; Ikeda & Nishinari, 2001b) for protein solutions at low shear rates, and have been have been..."

Repeated phrase deleted

(9) Regarding this paragraph: "The type of strain sweep behaviour seen for the WPC solutions is the so called type I behaviour as described by Hyun et al. (Hyun, Kim, Ahn & Lee, 2002). In type I behavior there is an LVER region, followed by decreasing G' above a critical strain (strain thinning behaviour). Type I behaviour is common in polymer solutions and its physical explanation is similar to that for shear thinning behaviour in solutions, i.e. at low shear rate/strain the generated stress is too low to disrupt the structure, but as the shear rate/strain increases proteins align along the flow direction. This type of strain sweep behaviour is illustrated in Figure 1, where the strain sweep for WPC at a concentration of 21% protein is plotted". The authors are not clear with these statements, since type I corresponds to classification of large amplitude oscillatory shear (LAOS) behavior. Hyun et al. (2002) stated that depending on the interactions between the microstructures, exist at least four types of LAOS behavior: type I, strain thinning (G' , G'' decreasing); type II, strain hardening (G' , G'' increasing); type III, weak strain overshoot (G' decreasing, G'' increasing followed by decreasing); type IV, strong strain overshoot (G' , G'' increasing followed by decreasing). This should be clearly stated in the manuscript in order to understand the concepts.

A section has been added on page 8/9 lines 156-162 to explain this.

(10) These can be rewritten as one sentence: "For WPC solutions of all concentrations the strain sweep data (not shown for protein concentrations 6-18%) is in agreement with previous results of other researchers (Ikeda & Nishinari, 2000; 2001a). For WPC a LVER was observed for all protein concentrations at low strains (below strain 0.02)".

Changed to "For WPC solutions of all concentrations the strain sweep data (not shown for protein concentrations 6-18%) an LVER was observed for all protein concentrations at low strains (below strain 0.02) in agreement with results of other researchers (Ikeda & Nishinari, 2000; 2001a)."

(10) The affirmation of this sentence is confusing: “This agrees with our previous results on the concentration dependence of shear viscosity (Zhang et al., 2016b) where we observed a constant shear viscosity at low shear rates that was independent of WPC concentration”; since in the present study the authors did not show the viscosity of these solutions.

Changes have been made in lines 148-151 to clarify this.

(11) The authors explained the results based on another paper, but they did not recall the terms, making it difficult to analyze: “At higher protein contents, the strain sweep behaviour changes to the type III or type IV behaviour described by Hyun et al. (2002).”

This has been addressed in the reply to comment 9.

(12) In Figure 2 both axis are in log scale, but this is not indicated in the plot: “As the protein concentration increases the elastic modulus G' starts to increase at a greater rate and follow a power law dependence, i.e. $G' \sim c^m$ where c is the protein concentration and m is the slope of the linear best fit lines in Figure 2”.

The legend to Figure 2 has been changed to highlight that this is a log-log plot.

(13) Figure 2 do not have a correct scale in the x axis. The y-axis is not the same, making difficult to compare.

The x-axis has been changed to include the minor axes labels. We disagree with the reviewer about the differing y-axis range. If we change this so that all plots in the figure have the same y-axis, we cannot see the biphasic nature of the plots. For this reason we have left these unchanged in the manuscript.

(14) The authors repeated the idea in this phrase: “The complex viscosity of PDWPC-A solutions (Figure 4) was found to be close to the shear viscosity at concentrations of 14% and 16%, indicating that these solutions followed the Cox-Merz rule and thus, aggregation of proteins was absent or was weak. At 18% protein and above deviation from Cox-Merz behaviour was seen at lower ω /shear rate, but Cox-Merz behaviour was seen at ω above about 1Hz. At the lower protein contents (14 & 16%) the Cox-Merz plots suggest liquid-like behaviour of PDWPC-A in solution, but at higher concentrations more structured elastic solutions are formed”. The idea is repeated, please rewrite the sentences: “This would indicate weak interparticle forces and thus fragile aggregates of proteins existing in these solutions. At 18% and 21% solutions of these proteins the complex viscosity is always higher than the shear viscosity suggesting the occurrence of strong interparticle forces and formation of strong aggregates of protein molecules in these solutions”.

This has been changed in the text on lines 319-323.

(15) These two ideas seem to be opposite, please clarify the meaning of DLA structure: “In DLA, particles collide and stick together in a rigid structure that does not rearrange with time. Aggregates of gels formed from this type of aggregation are open and diffuse, and are characterised by fractal dimensions around 1.8....(...). Aggregates formed via DLA have a more compact, dense...”.

This was a typographic error and should have said “Aggregates formed via RLA have a more compact, dense...”

This has been altered in the text on line 362,

(16) A parenthesis is missing in the equation 2.

This has been added.

(17) Have the authors performed the frequency sweep or the viscosity measure at pH4.5 for the other concentrations of products?

No these measurements were not made at lower protein concentrations.

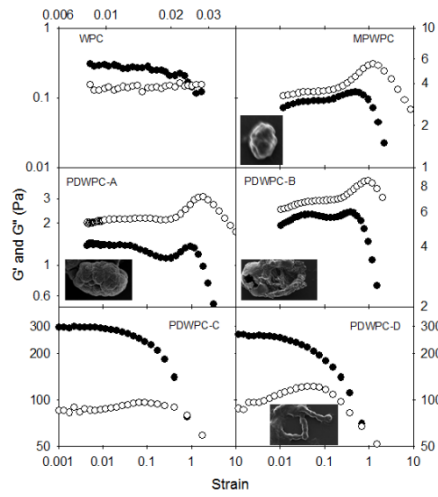
As the authors mention, the increase in G' and G'' with the decrease of the pH to 4.5 is due to the inhibition of electrostatic repulsion due to the charge screening in WPC aggregates. This process is called acid induced gelation or cold set gels (it can be also induced by salts), since gelation occurs at room temperature. This is important, but it is not mentioned in the manuscript. There are a lot of new articles with information that authors can compare in this section. With these techniques, gels of 2% of protein have been obtained for whey proteins.

We are well aware of the phenomenon of cold gelation and have mentioned this in the introduction section in the original manuscript. A further section from lines 534-546 has been added to discuss the similarities and differences with cold gelling WPC's. A further two references have been added to the reference list on lines 571-577.

Highlights

- PDWPCs can be classified based on the viscoelastic properties of their solutions
- PDWPCs with compact particles exhibit strong strain overshoot and form weak gels
- PDWPCs which have open, porous particles show weak strain overshoot
- PDWPCs with open particles form weak gels or strong gels depending on concentration
- All PDWPCs form fractal aggregates in solution

Graphical Abstract



1 Properties of Partially Denatured Whey Protein Products: Viscoelastic
2 Properties

3

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5 Stephen R. Euston^{1*}

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19

20 **Abstract**

21 Partially denatured whey protein products (PDWPC's) can be classified based
22 on the viscoelastic properties of their solutions. Strain sweeps show that
23 PDWPC-A and -B and microparticulated WPC (MPWPC) with compact,
24 spherical aggregated particles exhibit a strong strain overshoot. PDWPC-C
25 and -D, on the other hand, which have open, elongated porous particles show
26 a weak strain overshoot. The concentration dependence of the elastic modulus
27 G' in the linear viscoelastic region has a biphasic power law dependence with
28 concentration for all protein products studied, except for WPC where G' is
29 independent of protein concentration. Frequency sweeps suggest that MPWC
30 solutions form a strong physical gel at all concentrations above 14% (w/w).
31 PDWPC-A and -B form weak gels over the same concentration range.
32 PDWPC-C and -D also form weak gels at 14% protein (w/w) but strong
33 physical gels at higher concentrations. The frequency dependence of G' and
34 G'' for all aggregated proteins show a power law dependence indicating fractal
35 type structures. For all solutions above a critical concentration, the fractal
36 dimensions span the range 1.6-2.3, indicating a range of gel network
37 structures from open and diffuse to compact and dense. Adherence to the
38 empirical Cox-Merz rule was observed in PDWPC-A, -C and -D at
39 concentrations of 14 and 16% (w/w) protein, suggesting liquid-like behaviour.
40 At higher protein concentrations the deviations from the Cox-Merz rule suggest
41 more pronounced elasticity in the structure. For PDWPC-B, the behaviour is

42 complex, with deviation from the Cox-Merz rule at low frequencies/shear rates,
43 but correspondence at higher frequencies/shear rates at all concentrations.
44 This indicates a frequency-dependent change from liquid-like behaviour over
45 long timescale deformations, to a solid-like behaviour at short timescale
46 deformations. MPWPC solutions of all concentrations do not follow the Cox-
47 Merz rule, suggesting solid-like behaviour. The PDWPCs exhibit a complex
48 rheological behaviour which suggests they could be versatile thickening,
49 texturizing and fat replacement ingredients that will offer food manufacturers
50 greater control over the texture of formulated fluid foods.

51

52 **Keywords:** Partially denatured whey proteins; oscillatory rheology; frequency
53 sweep; fractal; Cox-Merz rule

54 **Introduction**

55 Heat-processed whey proteins have been investigated as thickening and
56 gelling ingredients in foods, and in particular as potential fat replacers
57 (McClements, Chung & Wu, 2017). It has been widely reported that whey
58 proteins that are partially denatured with pre-heat treatment at low ionic
59 strength exhibit gelling properties at room temperature and thus are defined as
60 cold-gelling whey protein concentrates (WPC's) (McClements & Keogh, 1995).
61 The cold-setting gelation could be of great value in the food industry, and
62 would meet the thickening requirement for some special foods that are not
63 suitable for heat processing.

64 In previous papers (Zhang, Arrighi, Campbell, Lonchamp & Euston, 2016a;
65 2016b) we reported on the manufacture of partially denatured whey protein
66 products (PDWPCs) that exhibit differing viscosity modifying properties
67 dependent on the method used for processing. More specifically, we showed
68 that by controlling the degree of disulphide bonding between aggregates (by
69 altering the temperature, pH and heating time) it was possible to form PDWPC
70 particles of differing degree of protein denaturation and particle size and
71 morphology (Zhang et al., 2016a). All PDWPCs gave solutions of increased
72 viscosity compared to the starting whey protein concentrate solution (Zhang et
73 al., 2016b). Particles with a high degree of denaturation and an open phase
74 separated morphology gave much higher viscosities than particles of a lower

75 degree of denaturation that had a more compact globule-like structure (Zhang
76 et al., 2016a). These differences in viscosity are believed to arise through
77 differences in the way the particles interact with the water hydration shell
78 around the proteins, and the interactions between the particles themselves. A
79 detailed study of the flow properties of PDWPCs revealed a complex
80 dependence of the shear viscosity on the particle morphology, protein
81 concentration in solution and shear rate (Zhang et al., 2016b). The surface
82 structure of protein particles also plays a role, with particles of similar size but
83 with a rougher surface giving a higher viscosity for the same protein
84 concentration. These studies indicated that by controlling the aggregated whey
85 protein particle structure through changes in processing conditions, it is
86 possible to make protein products with a range of viscosity modifying potential
87 that could find application in food systems. To understand better the rheology
88 of PDWPCs we have extended our studies to look at the viscoelastic
89 properties of their solutions, and in particular strain and frequency dependent
90 properties.

91 **4 Material and methods**

92 **Protein solutions**

93 Commercial protein products that we have characterised previously (Zhang et
94 al., 2016a) were used for these studies. A whey protein concentrate (WPC)
95 Lacprodan 87 (Arla Foods Ingredients, Denmark), a microparticulated WPC

96 (MPWPC) (Simplex[®] 100[E], CP Kelco UK Limited, UK) and a series of
97 partially denatured whey protein (PDWPC) products, labelled PDWPC-A,
98 PDWPC-B, PDWPC-C, and PDWPC-D (Nandi Proteins, UK) were dissolved in
99 deionized distilled water at room temperature to make solutions with protein
100 concentrations of 6%, 9%, 12%, 14%, 16%, 18% and 21% (w/w). The
101 solutions were stirred gently for at least 1 h to allow hydration of the proteins.
102 Previous results (Zhang et al., 2016b) have shown that hydration (as assessed
103 by change in the constant shear viscosity with time) is complete in under an
104 hour for all protein particles. The pH of all solutions was in the range 6.7-7.0
105 and the pH was not adjusted before testing. The same procedure was
106 repeated for a further set of 21% (w/w) protein solutions, except that acetic
107 acid (≥ 99.7 w/w %) (Fisher Scientific, USA) was added to adjust the pH to 4.5.
108 The processing conditions used to prepare the PDWPC's and their properties
109 have been reported by us elsewhere (Zhang et al., 2016a). Briefly, the
110 PDWPC's were processed at differing temperature/pH combinations, 73 °C/pH
111 6.5, 72.5 °C/pH 6.4, 72.5 °C/pH 7.0, 74 °C/pH 7.0 giving a degree of
112 denaturation of 65, 45, 51 and 98% for PDWPC-A, -B, -C and -D respectively.

113 **Oscillatory rheology**

114 Oscillation measurements were performed with controlled stress mode using a
115 Bohlin Gemini rheometer (Malvern Instruments, UK), with 4°/40 mm cone and
116 plate at a temperature of 20 °C. Prior to oscillatory testing, the linear

117 viscoelastic region (LVER) was found by amplitude sweep measurements
118 taken at a frequency of 1 Hz. A stress/strain combination in the LVER was
119 chosen to use for the frequency sweep measurements to ensure that during
120 these tests the stress and strain was maintained below that which would
121 disrupt the solid structure of the solutions.

122 Frequency sweep measurements were performed in the range from 0.001 Hz
123 to 15 Hz, so that the largest angular frequency, ω , is of the order of 100 rad/s.

124 Oscillatory measurements in the linear viscoelastic region are often employed
125 for the investigation of gel systems, since the solid and liquid responses to
126 shear stress can be distinguished (Clark, Kavanagh, & Ross-Murphy, 2001;
127 Ikeda & Nishinari, 2001a). Comparing the storage (G') and loss (G'') moduli
128 with each other allows to define a system as having solid-like ($G' > G''$), liquid-
129 like ($G' < G''$), or critical gel ($G' = G''$) behaviour (Barnes, 2000; Barnes, Hutton, &
130 Walters, 1989; Goodwin & Hughes, 2008; Macosko, 1994; Morrison, 2001).

131 Moreover, the relationship between the dynamic moduli (G' , G'') and the
132 observation time, (represented experimentally as the frequency, ω , of
133 oscillation) reveals the structure of the gels. Vilgis and Winter (1988), proposed
134 a power law relationship between G'/G'' and ω for self-similar or fractal
135 structures for chemical gels, which has been proven to be valid for physical
136 gels (Miyoshi & Nishinari, 1999).

137 **5 Results and discussion**

138 To obtain the dynamic viscoelastic properties, the linear viscoelastic region of
139 a material must be defined first, i.e. where linearity between strain and stress is
140 observed (Barnes, 2000; Barnes et al., 1989; Goodwin & Hughes, 2008;
141 Macosko, 1994; Morrison, 2001). Experimentally, this linear viscoelastic range
142 is obtained from strain sweep measurements (Ikeda & Nishinari, 2001a).
143 Representative strain sweeps at 21% protein content are plotted for all protein
144 products in Figure 1. For WPC solutions of all concentrations the strain sweep
145 data (not shown for protein concentrations 6-18%) an LVER was observed for
146 all protein concentrations at low strains (below strain 0.02) in agreement with
147 results of other researchers (Ikeda & Nishinari, 2000; 2001a). The G' was
148 always higher than G'' in these LVER regions, suggesting significant protein-
149 protein interaction and solid-like behaviour in this region. Ikeda & Nishinari
150 (2000; 2001a) explain this in terms of a colloidal crystal-like behaviour where
151 the proteins interact through long-range electrostatic interaction to form an
152 ordered structure. In a previous study on the concentration dependence of
153 shear viscosity in PDWPC solutions (Zhang et al., 2016b) we observed
154 constant shear viscosity at low shear rates that was independent of WPC
155 concentration. We interpreted this as evidence of ordered, solid-like colloidal
156 crystal phases. These have been observed by others (Matsumoto & Inoue,
157 1996; Ikeda & Nishinari, 2000; Ikeda & Nishinari, 2001a; Ikeda & Nishinari,
158 2001b) for protein solutions at low shear rates, and have been attributed to

159 long-range, but weak repulsive electrostatic interactions between proteins.

160 Hyun et al. (Hyun, Kim, Ahn & Lee, 2002) have defined at least four types of

161 large amplitude oscillatory strain (LAOS) behaviour which depend on the

162 interactions between the microstructure elements in the solution. These are

163 type I, strain thinning (both G' and G'' decreasing with increasing strain); type

164 II, strain hardening (both G' and G'' increasing); type III, weak strain overshoot

165 (G' decreasing, G'' first increases followed by a decrease); type IV, strong

166 strain overshoot (both G' and G'' increase followed by a decrease). The type of

167 strain sweep behaviour seen for the WPC solutions is the so-called type I

168 behaviour as described by Hyun et al. (2002). In type I behaviour there is an

169 LVER region, followed by decreasing G' above a critical strain (strain thinning

170 behaviour). Type I behaviour is common in polymer solutions and its physical

171 explanation is similar to that for shear-thinning behaviour in solutions, i.e. at

172 low shear rate/strain the generated stress is too low to disrupt the structure,

173 but as the shear rate/strain increases proteins align along the flow direction.

174 This type of strain sweep behaviour is illustrated in Figure 1, where the strain

175 sweep for WPC at a concentration of 21% protein is plotted.

176 MPWPC and the PDWPC solutions show a different type of strain sweep

177 behaviour compared to WPC. The protein products show a strain thinning

178 behaviour at lower protein contents (9% and below for MPWPC, 18% and

179 below for PDWPC-A, and 14% and below for PDWPC-B, PDWPC-C and

180 PDWPC-D, data not shown). At higher protein contents, the strain sweep

181 behaviour changes to the type III or type IV behaviour described by Hyun et al.
182 (2002). This type of behaviour is seen in Figure 1 where the strain sweep at
183 21% protein is plotted for all protein products. Type III behaviour, which is
184 shown by PDWPC-C and PDWPC-D is characterised by a local maximum in
185 the G'' curve (Figure 1) above the LVER. This is found, typically, in systems
186 such as concentrated emulsions (Bower, Gallegos, Mackley & Madiedo, 1999)
187 and dispersed hydrogel spheres (Wyss et al., 2007). This type of behaviour,
188 which is termed weak strain overshoot, is believed to be due to changes in the
189 structure of flocs of particles under the action of the oscillatory shear stresses,
190 although the exact mechanisms appear to depend on the nature of the system.
191 Raghavan and Khan (1995) explain the appearance of weak strain overshoot
192 as being due to an increase in the floc size at a critical stress/strain. Others
193 (Tirtaatmadja, Tam & Jenkins, 1997) suggest this is because of an increase in
194 the effective volume of the flocs. Parthasarathy & Klingenberg (1999) take a
195 slightly different view and believe the increase in G'' is due to small
196 rearrangements of floc structure followed by larger rearrangements that lead to
197 the decrease in G'' again at larger strains. Type IV behaviour, or strong strain
198 overshoot, is displayed by MPWPC, and PDWPC's A and B and is
199 characterised by a maximum in both G'' and G' (Hyun et al., 2002). This is
200 believed to arise when there are relatively strong inter-particle interactions
201 such as hydrophobic association. These results suggest that the MPWPC and
202 PDWPC's A and B have stronger inter-particle interactions in solution

203 compared to PDWPC C and D, even though the plateau G' values are lower.
204 These results agree with our earlier study of the shear viscosity behaviour of
205 the protein products which showed that MPWPC, PDWPC-A and PDWPC-B
206 have a strong time dependence for viscosity recovery from a step change in
207 shear rate from 1 s^{-1} to 100 s^{-1} back to 1 s^{-1} (Zhang et al., 2016b), whereas
208 PDWPC's C and D recover quickly. This again also indicates stronger
209 interactions in the MPWPC and PDWPC's A and B that are slow to reform
210 when high shear is removed. To explain the higher G' in the LVER and the
211 higher viscosity for a given shear rate (Zhang et al., 2016b) for PDWPC's C
212 and D, it should be noted that these have a much larger particle size than the
213 other aggregated proteins, and for PDWPC-D we have observed a very open,
214 porous particle structure (Zhang et al., 2016a). This, we believe, leads to a
215 greater hydrodynamic effect on the viscosity than MPWPC and PDWPC's A
216 and B, even though the latter apparently have stronger interactions in solution.
217 Therefore, in PDWPC-C and D solutions hydrodynamic interactions must be
218 dominating over other types of inter-particle interaction. The aggregated
219 protein products also differ from WPC solutions in terms of the relative
220 magnitude of the G' and G'' . For MPWPC, and PDWPC's A and B the G'' is
221 higher than G' at all protein concentrations, suggesting that the particle
222 solutions dissipate the applied strain mainly through viscous flow rather than
223 storing the applied strain as elastic energy. This is the case also for PDWPC-C
224 and D at the lower solution concentrations (14% and below for both), with a

225 change to elastic response (G') dominating over viscous flow (G'') at higher
226 concentrations.

227 The concentration dependence of the constant plateau value of G' in the linear
228 viscoelastic region for the different protein solutions is shown in Figure 2. This
229 figure shows that for WPC the G' in the LVER is independent of protein content
230 which was consistent with the findings of Lizarraga et al. (Lizarraga, De Piante
231 Vicin, González, Rubiolo & Santiago, 2006). We also observed the same
232 independence of shear viscosity on protein content for WPC at low shear rates
233 in our previous paper (Zhang et al., 2016b). The aggregated protein products
234 all show a weak dependence of G' on concentration at the lower protein
235 concentrations. As the protein concentration increases the elastic modulus G'
236 starts to increase at a greater rate and follow a power law dependence, i.e. G'
237 $\sim c^m$ where c is the protein concentration and m is the slope of the linear best
238 fit lines in Figure 2. These power law trends at high protein concentrations
239 suggest increased interactions between protein molecules. It was also found
240 that the concentration dependence, i.e. the values of m , as shown in Table 1,
241 of MPWPC and PDWPC-A and PDWPC-B were much smaller than those of
242 PDWPC-C and PDWPC-D. This suggests weaker structure formed by the
243 former ones, while much stronger aggregates, such as network or physical gel
244 existed in the latter ones (Xu et al., 2013). Others have observed a power law
245 relationship between elastic modulus and concentration for both polymer
246 solutions and protein gels. The value of the exponent m varies between protein

247 types. Ferry (1948, 1980) found a value of $m=2$ for gelatin with higher values in
248 the range $m = 2-7$ found for other protein gels (Bikbow, Grinberg, Antonov,
249 Tolstoguzov & Schmandke, 1979; Clark, 1989; Kohyama, et al. 1992; Tokita et
250 al. 1985; Vreeker et al. 1992). This includes $m= 5$ for soybean globulins
251 (Bikbow et al. 1979), $m = 3.4$ for soybean 11S globulin (Kohyama & Nishinari,
252 1992), $m = 4$ for casein (Tokita, Niki & Hikichi, 1985), and $m = 4.1$ for whey
253 protein isolate (Vreeker, Hoekstra, Den Boer & Agterof, 1992). From Table 1 it
254 can be seen that the MPWPC and PDWPC-A and PDWPC-B solutions studied
255 here fall within this range of $m=2-7$, but PDWPC-C and PDWPC-D have higher
256 values of m suggesting the latter form gels that are more strongly associated in
257 solution than corresponding heat set WPC gels. Also presented in Table 1 is
258 the crossover concentration, at which the G' starts to increase rapidly in Figure
259 2. Presumably, this crossover concentration corresponds to a change in the
260 interaction between the aggregated protein particles, and may correspond to a
261 concentration where hydrodynamic interactions between the particles become
262 important. We also observed a crossover concentration for the equilibrium
263 shear viscosity (Zhang et al. 2016b) at high (100 s^{-1}) and low (0.001 s^{-1}) shear
264 rates, which occurred at similar concentrations.

265

266 To probe the oscillatory response of the solutions of the aggregated protein
267 samples further, frequency sweep measurements were employed. These
268 measurements were carried out for solutions of concentration above 14% w/w.

269 In Figures 3-7 frequency sweeps and Cox-Merz plots of different protein
270 solutions are shown. Frequency sweeps determine how the material responds
271 to a constant deformation applied over different timescales. Since the time
272 over which the oscillation is applied is equal to the reciprocal of the frequency,
273 low frequency measurements correspond to long time (slow) deformation, and
274 high frequencies to short time (rapid) deformations. The frequency (ω)
275 dependence of G' and G'' can give an indication of the type of structure present
276 in the solution. At strains in the LVER region, the frequency sweep can be
277 classified into three general types. Weak gels, such as entangled polymer
278 solutions exhibit a strong dependence of G' with ω , and can exhibit a
279 crossover of the G' and G'' curves indicating a change between solid-like
280 behaviour and liquid-like behaviour. Strong, cross-linked gels have only weak
281 dependence of G' on ω and G' always greater than G'' . In between these two
282 types are the physical gels with some frequency dependence of G' but no
283 G'/G'' crossover. Based on these criteria we can classify the gel behaviour of
284 our aggregated whey protein products. For MPWPC, it is found that G' is
285 always larger than G'' at all protein concentrations studied (Figure 3).
286 Furthermore, the G' has a relatively weak dependence on ω . This suggests the
287 formation of a solid-like physical gel of the microparticulated proteins.
288 PDWPC's A and B (Figures 4 & 5) show a different type of behaviour. Both
289 show a greater dependence of G' on ω than MPWPC. In addition, for PDWPC-
290 A the G'' is always higher than G' , other than at 21% (w/w) where the two

291 moduli are virtually the same at all frequencies. Similarly, for PDWPC-B the G''
292 is higher than G' up to 21% protein, at which concentration G' now dominates
293 over G'' . This behaviour is more characteristic of a weak gel network. With
294 PDWPC-C and PDWPC-D the type of gel formed depends on concentration
295 (Figures 6 & 7). At 14% both PDWPC-C and PDWPC-D form a weak gel
296 (relatively strong dependence of G' on ω , with some evidence of a G'/G''
297 crossover), but formation of physical gels at higher concentrations where G' is
298 always significantly higher than G'' .

299 The Cox-Merz rule is an empirical observation (Cox & Merz, 1958) that for
300 isotropic fluids there is direct correspondence between the complex viscosity
301 measured in a frequency sweep, and the shear viscosity measured as a
302 function of shear rate. Young (2015) states that this means that a fluid that is
303 predominantly a liquid (Newtonian) will obey the Cox-Merz rule, whilst those
304 that are elastic, or Non-Newtonian fluids will not. Thus, adherence (or
305 otherwise) to the Cox-Merz rule can be used to assess further the structure in
306 a system.

307 Plots of complex viscosity and shear viscosity for the MPWPC solutions
308 (Figure 3) show deviation from the Cox-Merz rule at all concentrations, with
309 complex viscosity higher than shear viscosity at all shear rates and
310 frequencies ω . Such behaviour suggests that the solid-like structure formed in
311 these solutions is susceptible to disruption at large strains (Ikeda & Nishinari,
312 2001a; Lizarraga et al., 2006). Renard *et al.* (Renard, Robert, Faucheron &

313 Sanchez, 1999) observed similar phenomenon in MPWPC and suggested that
314 it was due to flocculation of the microparticulated proteins at low shear
315 stresses and disruption of these flocs by large shear stresses and strains.

316 The complex viscosity of PDWPC-A solutions (Figure 4) was found to be close
317 to the shear viscosity at concentrations of 14% and 16%, indicating that these
318 solutions followed the Cox-Merz rule and thus, aggregation of proteins was
319 absent or was weak. This would indicate weak interparticle forces and thus
320 fragile aggregates of proteins existing in these solutions. At 18% and 21%
321 solutions of these proteins the complex viscosity is always higher than the
322 shear viscosity suggesting the occurrence of strong interparticle forces and
323 formation of strong aggregates of protein molecules in these solutions.. The
324 Cox-Merz behaviour for PDWPC-B is also complex (Figure 5). At all
325 concentrations deviation from Cox-Merz behaviour is seen at lower shear
326 rate/ ω , but at higher shear rate/ ω convergence of complex and shear viscosity
327 is observed, suggesting solid-like behaviour over long time scale deformations
328 and liquid-like behaviour at short timescale deformations.

329 Adherence to the empirical Cox-Merz rule was observed only for the 14% and
330 16% solutions of PDWPC-C and PDWPC-D, although as discussed for
331 PDWPC-A and PDWPC-B there was some divergence of shear viscosity from
332 complex viscosity at low shear rates/ ω . This would indicate weak interparticle
333 forces and thus fragile aggregates of proteins existing in these solutions. At
334 18% and 21% solutions of these proteins the complex viscosity is always

335 higher than the shear viscosity suggesting the occurrence of strong
336 interparticle forces and formation of strong aggregates of protein molecules in
337 these solutions. Large increases were observed in G' , G'' , and η^* for the
338 PDWPC-C and PDWPD solutions above 18%, which were much larger than
339 those of MPWPC, PDWPC-B and PDWPC-B at the same concentrations,
340 suggesting a strong network or physical gel formed by the aggregated protein
341 particles.

342 The structure of the MPWPC and PDWPC solutions can be further probed by
343 following the frequency dependence of G' and G'' . Close to the gelation point
344 of branched polymers both G' and G'' follow the scaling rule G' or $G'' \sim \omega^n$ and
345 the complex viscosity $\eta^* \sim \omega^{n-1}$ (Muthukumar, 1989). Several attempts have
346 been made to relate the exponent n to the structure or fractal dimension of
347 gels. The fractal dimension is often used to characterise the structure of
348 aggregated or gelled systems (Hagiwara, Kumagai, Matsunaga & Nakamura,
349 1997). Computer simulations have been used to establish the theoretical
350 fractal dimension (d_f) value for two limiting forms of aggregation – diffusion
351 limited (DLA) and reaction limited (RLA) (Witten & Sander, 1981; Weitz,
352 Huang, Lin & Sung, 1985). In DLA, particles collide and stick together in a rigid
353 structure that does not rearrange with time. Aggregates of gels formed from
354 this type of aggregation are open and diffuse, and are characterised by fractal
355 dimensions around 1.8. In RLA, on the other hand, particles aggregate more
356 loosely, and the aggregates formed are able to rearrange over time.

357 Aggregates formed via RLA have a more compact, dense structure with a
358 fractal dimension of around 2.1. The value of $d_f = 1.8$ is the lower practical limit
359 for 3-dimensional systems. The upper limit for d_f is the Euclidian dimensionality
360 of the system, i.e. 3 for 3-D systems and 2 for 2-D systems. Percolation theory
361 predicts that (Stauffer, 1985),

$$362 \quad n = \frac{d}{d_f + 2} \quad (1)$$

363 where d is the Euclidian dimensionality of the system ($d=3$ in this case) and d_f
364 the fractal dimension. For percolation theory, $d_f = 3$ corresponds to $n=0.6$, and
365 we would expect fractal gels to have $n>0.6$. Muthkumar (1989) however, found
366 very small values of n for polymer solutions close to the gel point, which would
367 give unrealistically high (>3) d_f values based on percolation theory (eqn. 1). Doi
368 & Edwards, (1986) explained this as being due to the screening of the
369 excluded volume effects due to high concentration of polymers. Muthkumar
370 (1989) derived an alternative relationship for n , taking account of screening
371 excluded volume effects, as,

372

$$373 \quad n = \frac{d(d + 2 - 2d_f)}{2(d + 2 - d_f)} \quad (2)$$

374

375 The value of n for the scaling of G' , G'' and η^* for the MPWPC and PDWPC's
376 was calculated and is presented as an average of the three in Table 2 along
377 with the calculated average d_f . From the discussion above, we would expect d_f
378 to fall approximately within the range 1.8 - 2.1. Values of d_f lower than 1.8 have

379 been observed for fibrillar aggregates formed by polyvinyl chloride (Dahmani,
380 Fazel, Munch & Guenet, 1997) where the aggregate takes a more linear form.
381 Similar fibrillar gels are not unknown for proteins (Nicolai & Durand, 2013). For
382 protein gels, d_f higher than 2.1 but lower than 3 is not uncommon at higher
383 protein concentrations as the packing density of the molecules can be high,
384 and restructuring of the gel after formation is common (Eleya, Ko &
385 Gunasekaran, 2004). Percolation theory assumes that polymer solutions show
386 fractal properties close to the percolation threshold, i.e. the concentration at
387 which a percolating network just forms. When fractal dimension is calculated
388 using percolation theory (eqn. 1) for all samples, a wide range of d_f values are
389 found. For MPWPC, the d_f values are all $\gg 3$. This suggests that at 14%
390 MPWPC and above we are above the percolation threshold and so percolation
391 theory does not hold. If we look at the crossover concentration (m) in Table 1,
392 we see that this is about 12%. Therefore it is not unreasonable to suggest that
393 the crossover concentration in Figure 2 might correspond to the percolation
394 threshold, explaining why d_f is indeterminate above the crossover
395 concentration. A similar result is observed for PDWPC-C and PDWPC-D
396 where d_f calculated from eqn.1 are large at all concentrations of 14% and
397 above, suggesting the solutions are above the percolation threshold. Again,
398 from Table 1 we see that the crossover concentration for these two protein
399 products is 12%, i.e. below the concentrations where d_f is calculated in Table
400 2. For PDWPC-A and PDWPC-B the results are more complex. For PDWPC-A

401 the crossover concentration in Table 1 is 16%. At 16% PDWPC-A solutions
402 and below, the d_f calculated from percolation theory is less than 1.8, and above
403 16% it is high and close to 3. This again suggests that percolation theory does
404 not hold for PDWPC-A at these concentrations and that the percolation
405 threshold is between 16-18%. Similarly, for PDWPC-B the observed crossover
406 concentration is also 16%. In this case, the 16% solution of PDWPC-B gives a
407 sensible value for d_f calculated from eqn. 1, but below this concentration $d_f <$
408 1.8 and above 16% it is > 3 . These results suggest that the percolation
409 threshold for PDWPC-B solutions is close to 16%, thus adding more weight to
410 the supposition that the crossover concentration is equivalent to the
411 percolation threshold.

412 If screening of the excluded volume contributions is assumed in these
413 solutions, and Muthkumar's equation for calculating d_f is used (Muthkumar,
414 1989) more sensible value of fractal dimension are found at concentrations
415 close to and above the crossover concentration. For MPWPC eqn. 2 yields d_f
416 values of 2.1-2.2 at all concentrations, values that are close to that for RLA.
417 Winter and Chambon (1986) and Vilgis and Winter (1988) proposed the power
418 law exponent and the fractal dimension for a critical gel where the network
419 expanded to infinite are 0.50 and 2.0. This suggests that MPWPC solutions
420 form a fractal network at all concentrations studied. This would be consistent
421 with the observation from our previous work that MPWPC solutions contain
422 large flocs of smaller particles that would show fractal behaviour over a wide

423 range of length scales and concentrations both above and below the crossover
424 concentration. For PDWPC-A and PDWPC-B eqn. 2 predicts low d_f values
425 below the crossover concentration, and value around 1.6-1.7 above this.
426 Below the crossover concentration, it seems likely that open extended
427 structures form by association between PDWPC-A and PDWPC-B particles.
428 PDWPC-A and PDWPC-B (and MPWPC) also showed strong strain overshoot
429 indicating strong interactions between the particles, and this may lead to the
430 tendency for more extended aggregates below the crossover concentration.
431 For PDWPC-C and PDWPC-D the solutions exhibit fractal structures that
432 become more compact (d_f increases from 1.6 to 2.3 for PDWPC-C and from
433 1.9 to 2.3 for PDWPC-D) with increasing concentration. This suggests that a
434 more rubber-like (Macosko, 1994; Rao, 2007) and denser structure (Ikeda &
435 Nishinari, 2001a; Vilgis & Winter, 1988) forms at higher protein concentrations.
436 Invalidity of the power law was also found at low frequencies for both of
437 PDWPC-C and PDWPC-D gels, thus indicating that the network of the proteins
438 had a finite length scale. According to the interpretation of mechanical spectra
439 by Vilgis & Winter (1988) the frequency, ω , of the oscillations can be related to
440 the length scale, L of the fractal network as $\omega^{-1} \propto t \propto L^d$, where t is the
441 time for relaxation and d is determined by diffusion of the polymers.
442 Consequently, a limiting characteristic frequency, which defines the largest
443 length scale of the gel, can be obtained. This is defined by the frequency at
444 which linear scaling of G'/G'' with ω stops/starts. From Figures 6 and 7 the

445 characteristic frequencies for PDWPC-C and PDWPC-D were found to be the
446 same at 0.02 rad/s, indicating the same length scale of the self-similar network
447 of these two denatured protein solutions.

448 Many foods systems are acidic in pH, a situation that leads to inhibition of
449 growth of spoilage bacteria. The pH also has an effect on protein as they
450 contain acidic and basic amino acid groups, and at a certain pH, the isoelectric
451 point or pI, the net charge they have is neutral. Therefore, the pH might be
452 expected to influence the interactions and structure of solutions of proteins and
453 partially denatured proteins. Since WPC is a mixture of proteins containing
454 mainly β -lactoglobulin as well as α -lactalbumin and bovine serum albumin
455 (BSA), each of which have different isoelectric points (Bryant & McClements,
456 1998; de Wit & Klarenbeek, 1984; Kinsella & Whitehead, 1988), a definition of
457 the isoelectric point (pI) of WPC is complicated. Marinova *et al.* (2009)
458 proposed a concept of 'effective' pI for WPC and suggested that it is about 4.2
459 where the ζ -potential of the proteins is zero. In previous work we have
460 measured the pI of Lacprodan 87 as 4.5. To test the effect of acidic pH on the
461 MPWPC and PDWPC solutions, acetic acid was added to lower the pH to 4.5
462 to approach the pI of proteins and decrease the charges on the protein
463 surface.

464 The effect of lowering the pH on G' and G'' of the PDWPCs at a concentration
465 of 21% (w/w) are illustrated in Figure 8. Both G' and G'' increased for all
466 samples when the pH was reduced to the pI, suggesting strengthening of the

467 structure and increased interaction between protein particles, most likely from
468 a reduction in electrostatic repulsion (Elofsson, Dejmek, Paulsson & Burling,
469 1997; Goodwin & Hughes, 2008; Ikeda, Foegeding & Hagiwara, 1999). For
470 MPWPC, G' is always higher than G'' at pH 4.5, and the dependence of G' and
471 G'' on ω is relatively weak as it was at the higher pH (Figure 3) indicating that
472 the solid-like physical gel structure of the MPWPC is maintained at pH 4.5.
473 Cox-Merz plots for MPWPC at pH 4.5 show that the solutions do not adhere to
474 the Cox-Merz rule, the same as at the higher pH, with the complex viscosity
475 higher than the shear viscosity at all frequencies. The lack of adherence to the
476 Cox-Merz rule indicates stronger interfloc forces, and thus, stiffer aggregating
477 structure of MPWPC at low pH (Macosko, 1994; Rao, 2007). Both the complex
478 and shear viscosity at pH 4.5 are higher than that at the higher pH.

479 For PDWPC A and PDWPC B there is a weaker dependence of G' on ω at pH
480 4.5, and G' is always greater than G'' suggesting a strengthening of the gel
481 structure (Figure 8). The Cox-Merz rule solutions is also not followed for
482 PDWPC A and PDWPC B at pH 4.5 (Figure 9), whereas the rule is followed at
483 higher pH. More obvious violations of the Cox-Merz rule are seen for the acidic
484 solutions compared to those at natural pH of the protein solution, where the
485 violations are only present at low frequencies and shear rates. Therefore,
486 strain- susceptible structure of protein aggregates could be hypothesised to
487 form in the acidic solutions (Ikeda & Nishinari, 2001a). The complex viscosity at
488 pH 4.5 is always higher than at the higher pH for both PDWPC A and B, but the

489 shear viscosity shows unusual behaviour. For both PDWPC A and B, the
490 shear viscosity at pH 4.5 at low shear rates is higher than at the higher pH
491 (Figure 9). However, as shear rate increases the shear viscosity for PDWPC A
492 becomes the same as that at the higher pH, but for PDWPC B there is a
493 crossover and shear viscosity at higher shear rates is lower for pH 4.5. A
494 possible explanation for this is that as well as altering the interactions between
495 particles, a lower pH also causes changes to the internal structure of the
496 particles for PDWPC A and B. It is possible that the particle swells at low pH
497 and become more sensitive to disruption due to high shear rates. At pH 4.5 the
498 proteins will be close to the isoelectric point, and the proteins may flocculate in
499 the absence of electrostatic repulsion. These flocs are likely to be shear
500 sensitive, and will break up at higher shear rates leading to shear thinning
501 behaviour.

502 For both PDWPC-C and PDWPC-D both G' and G'' increase at the lower pH.
503 PDWPC-C and PDWPC-D exhibited larger complex viscosity at pH of 4.5 than
504 at natural pH, while there was no change in the shear viscosity, except at low
505 shear rates, where aggregates of proteins were supposed to be formed and
506 incompletely disrupted (Renard et al., 1999). Such observations suggest that
507 the gel formed by polymeric denatured protein is strengthened at pH 4.5 since
508 the electrostatic repulsions were reduced, but the flowing units remained the
509 same as those at natural pH when the gel structure was disrupted by large
510 strains.

511 A power law was found to hold for the solutions of all PDWPC solutions at pH
512 4.5, but with smaller values of n (Table 2) and higher fractal dimensions. The
513 lower values of n (slope) of the G' vs ω graphs (Figure 8) at pH 4.6 indicate a
514 reduced frequency dependence of G' and reveal a more solid-like behaviour of
515 the solutions (Goodwin & Hughes, 2008; Macosko, 1994; Rao, 2007). These
516 more solid-like properties could be due to the denser packing of the proteins in
517 the solutions.

518 Since there is no net charge on the protein surfaces at pI , hydrophobic
519 interactions and van der Waals attractions will predominate the electrostatic
520 repulsions between protein molecules and flocs, which causes the further
521 aggregation and flocculation of proteins (Ikeda et al., 1999; Verheul, Pedersen,
522 Roefs, & de Kruif, 1999). Increased hydrodynamic interactions between
523 protein flocs with larger volumes at pH of 4.5 would account for the increases
524 in viscosity of MPWPC and PDWPC solutions.

525 The phenomenon observed here is similar to cold gelation of whey proteins
526 observed by other researchers (Bryant & McClements, 1998). These are
527 produced by heating under similar conditions to the PDWPC's (pH close to 7,
528 temperatures above 70 °C) at low salt and low protein concentrations to
529 prevent protein aggregation and gelation (Barbut & Foegeding, 1993). The
530 solutions of these proteins can be induced to thicken or gel by addition of salts
531 (Barbut & Foegeding, 1993), or reduction of pH (Alting et al, 2004). The critical
532 difference between previously reported cold-gelling WPCs and the PDWPC's

533 studied here is that higher protein concentrations (20-26% total solids) can be
534 processed compared to cold-gelling WPCs. This is due to monitoring and
535 control of the denaturation process that ensures over aggregation and gelation
536 does not occur. This makes the process economically feasible for production
537 of PDWPC's.

538 **Conclusion**

539 The viscoelastic properties of solutions of PDWPC aggregates exhibit a
540 complex dependence on the protein concentrations and microstructure of the
541 particles. The solution behaviour of PDWPC's differs from and is more
542 complex than both WPC and MPWPC, differences that can be explained by
543 structural differences between MPWPC and PDWPC protein particles that lead
544 to differences in the way they interact. Aggregated particles with open,
545 fibril/tubule-like structures, such as found in PDWPC-D have more solid like
546 behaviour, whilst compact aggregates such as found in PDWPC-A and -B
547 have a more liquid-like structure. The rheological behaviour of the PDWPCs is
548 strongly concentration and frequency dependent, with changes between more-
549 liquid like and more solid-like behaviour occurring as protein concentration
550 increases, and with a change from long timescale (low frequency) to short
551 timescale (high frequency) deformations. The PDWPCs exhibit a complex
552 rheological behaviour which suggests they could be versatile thickening,
553 texturizing and fat replacement ingredients that will offer food manufacturers

554 greater control over the texture of formulated fluid foods.

555

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713 **Table Legends**

714 **Table 1** – Power law exponent, m , for the concentration dependence of G' for
715 different protein samples.

716 **Table 2** – Power law exponent, n , from the scaling of G' , G'' or η^* with
717 frequency (ω) and the fractal dimension percolation theory (eqn. 1) or eqn. 2.

718

719 **Figure Legends**

720 **Figure 1** - Strain dependence measured at 1 Hz of elastic, G' (●), and
721 viscous, G'' (○) moduli of protein product solution at a protein concentration of
722 21% (w/w).

723 **Figure 2** Log-log plot of the concentration dependence of G' in linear
724 viscoelastic region for WPC, MPWPC, PDWPC-A, PDWPC-B, PDWPC-C,
725 PDWPC-D solutions

726 **Figure 3** – Frequency sweep and Cox-Merz plots of MPWPC solutions.

727 **Figure 4** - Frequency sweep and Cox-Merz plots of PDWPC-A solutions.

728 **Figure 5** - Frequency sweep and Cox-Merz plots of PDWPC-B solutions.

729 **Figure 6** - Frequency sweep and Cox-Merz plots of PDWPC-C solutions.

730 **Figure 7** - Frequency sweep and Cox-Merz plots of PDWPC-D solutions.

731 **Figure 8** - Comparison of the frequency dependence of G' and G'' for PDWPC
732 solutions (21% w/w) at pH4.5 and at the natural pH of the solution.

733 **Figure 9** - Comparison of the shear rate dependence of shear viscosity (η) and
734 frequency dependence of complex viscosity (η^*) for PDWPC solutions (21%
735 w/w) at pH4.5 and at the natural pH of the solution.

736

738 **Table 1**

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	Concentration dependence (<i>m</i>)		Crossover concentration (<i>C</i> [*]) (%)
	Above <i>C</i> [*]	Below <i>C</i> [*]	
WPC	-	-	-
MPWPC	0.59	5.68	12
PDWPC-A	0.47	5.90	16
PDWPC-B	0.60	6.05	16
PDWPC-C	0.40	12.04	12
PDWPC-D	0.47	12.90	12

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741 **Table 2**

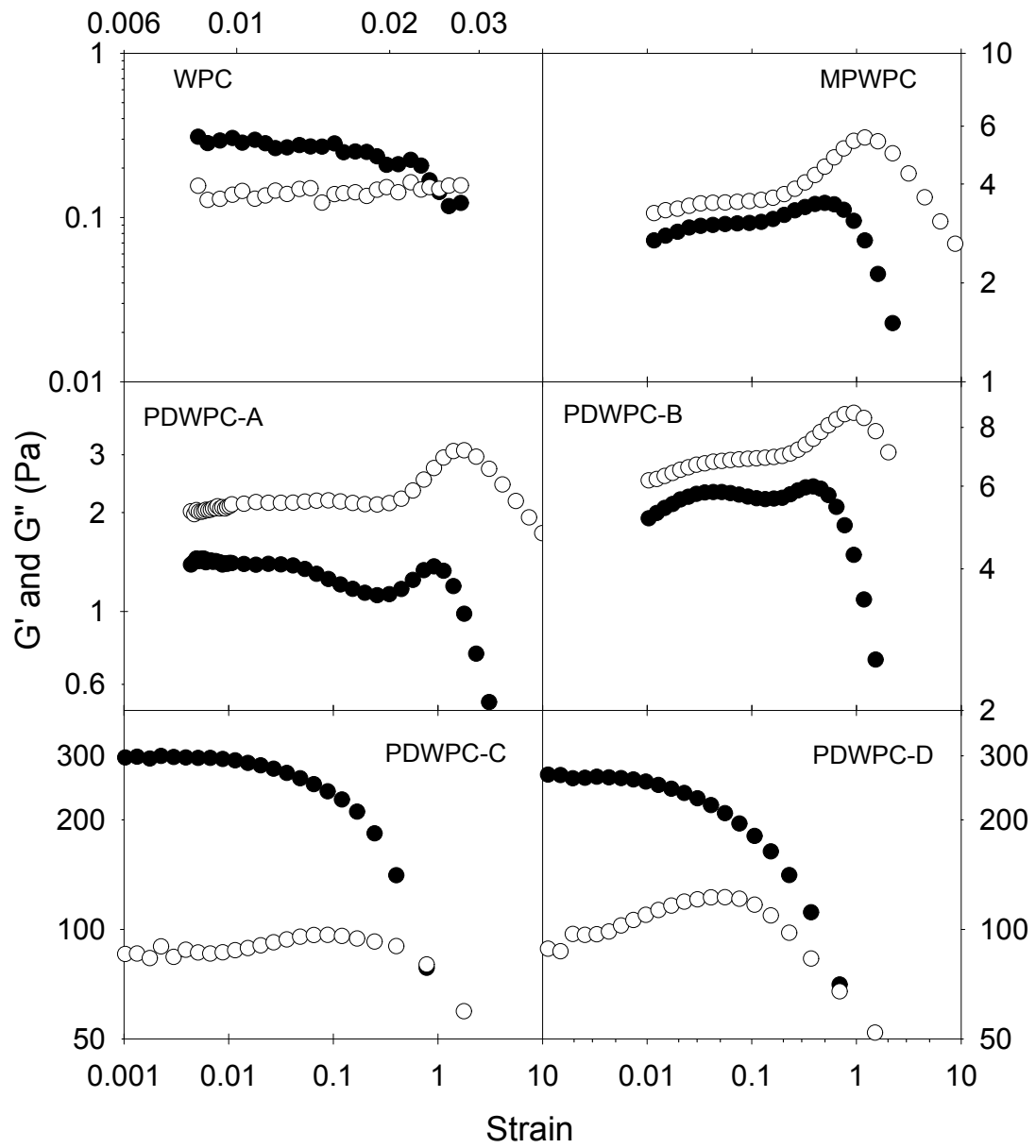
Protein Product	Mean n	d_f Percolation theory (Stauffer, 1989)	d_f Screened excluded volume (Muthkumar, 1989)
MPWPC			
14%	0.20 ± 0.10	14.6 ± 4.8	2.2 ± 0.1
16%	0.20 ± 0.00	13.0 ± 3.0	2.2 ± 0.1
18%	0.20 ± 0.10	13.8 ± 5.4	2.2 ± 0.1
21%	0.30 ± 0.10	9.3 ± 2.7	2.1 ± 0.1
pH4.5 (21%)	0.19 ± 0.03	-	2.3 ± 0.0
PDWPC-A			
14%	1.18 ± 0.34	1.0 ± 0.7	1.0 ± 0.4
16%	0.95 ± 0.22	1.3 ± 0.7	1.1 ± 0.4
18%	0.62 ± 0.02	2.8 ± 0.1	1.6 ± 0.0
21%	0.60 ± 0.02	3.0 ± 0.2	1.7 ± 0.0
pH4.5 (21%)	0.27 ± 0.03	-	2.1 ± 0.0
PDWPC-B			
14%	0.85 ± 0.08	1.5 ± 0.3	1.3 ± 0.1
16%	0.73 ± 0.06	2.1 ± 0.3	1.5 ± 0.1
18%	0.57 ± 0.03	3.3 ± 0.3	1.7 ± 0.0
21%	0.36 ± 0.04	6.5 ± 0.9	2.0 ± 0.1
pH4.5 (21%)	0.14 ± 0.01	-	2.3 ± 0.0
PDWPC-C			
14%	0.47 ± 0.01	4.4 ± 0.1	1.9 ± 0.0
16%	0.31 ± 0.02	7.6 ± 0.5	2.1 ± 0.0
18%	0.17 ± 0.00	15.6 ± 0.0	2.3 ± 0.0
21%	0.19 ± 0.01	14.1 ± 0.5	2.3 ± 0.0
pH4.5 (21%)	0.13 ± 0.01	-	2.3 ± 0.0
PDWPC-D			
14%	0.63 ± 0.02	2.8 ± 0.2	1.6 ± 0.0
16%	0.33 ± 0.04	7.2 ± 1.0	2.1 ± 0.1
18%	0.27 ± 0.02	9.3 ± 0.9	2.2 ± 0.0
21%	0.19 ± 0.02	13.6 ± 1.2	2.3 ± 0.0
pH4.5 (21%)	0.16 ± 0.01	-	2.3 ± 0.0

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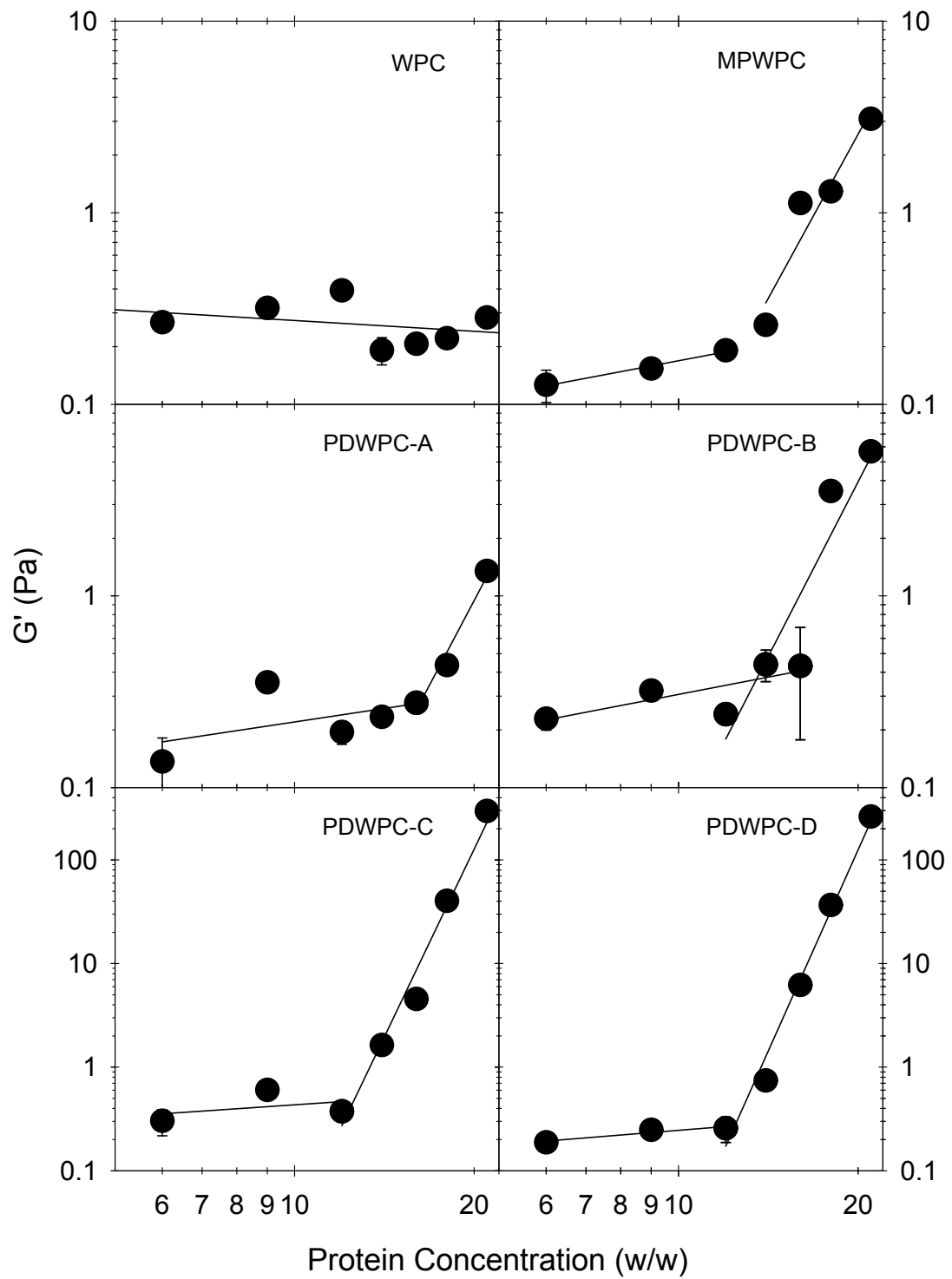
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Figure 1

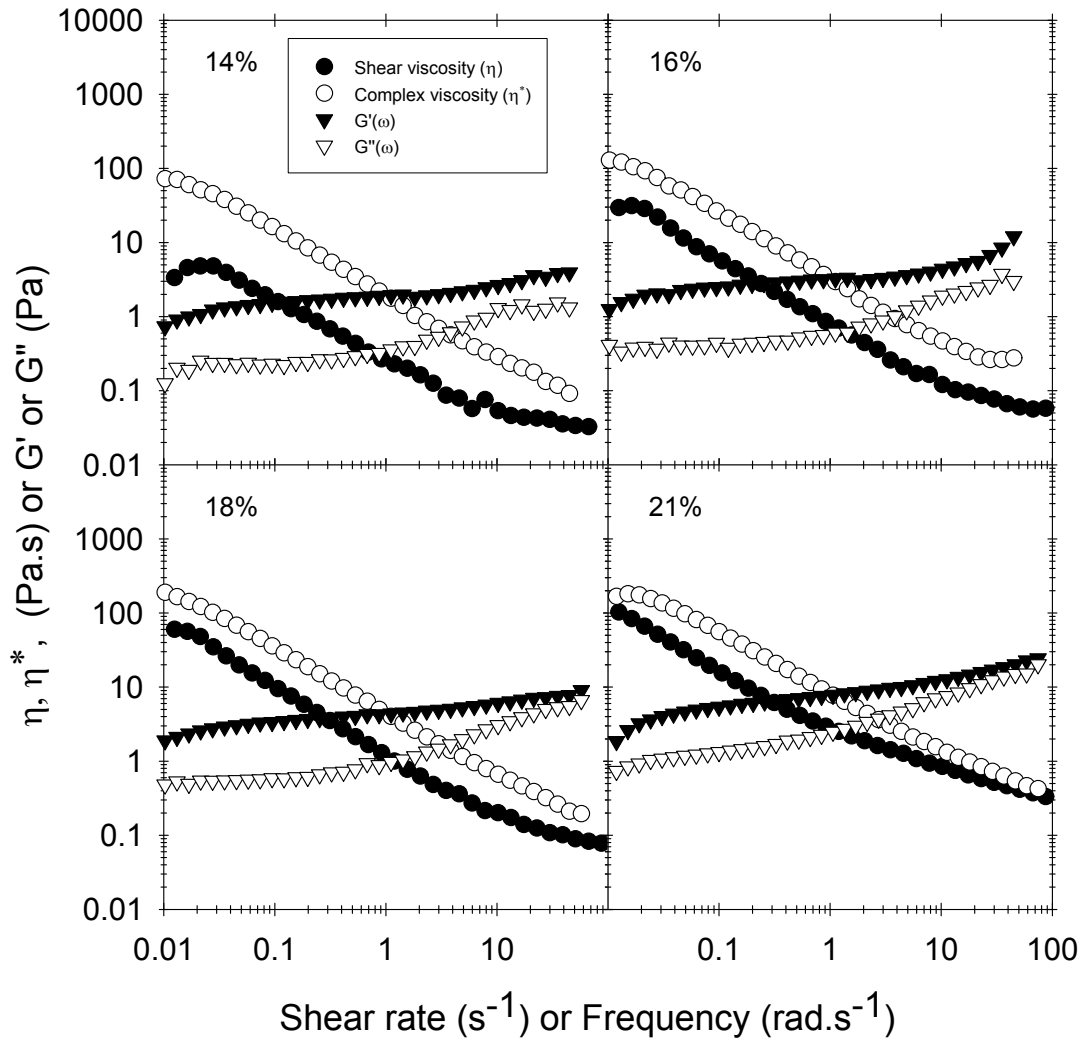


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Figure 2.

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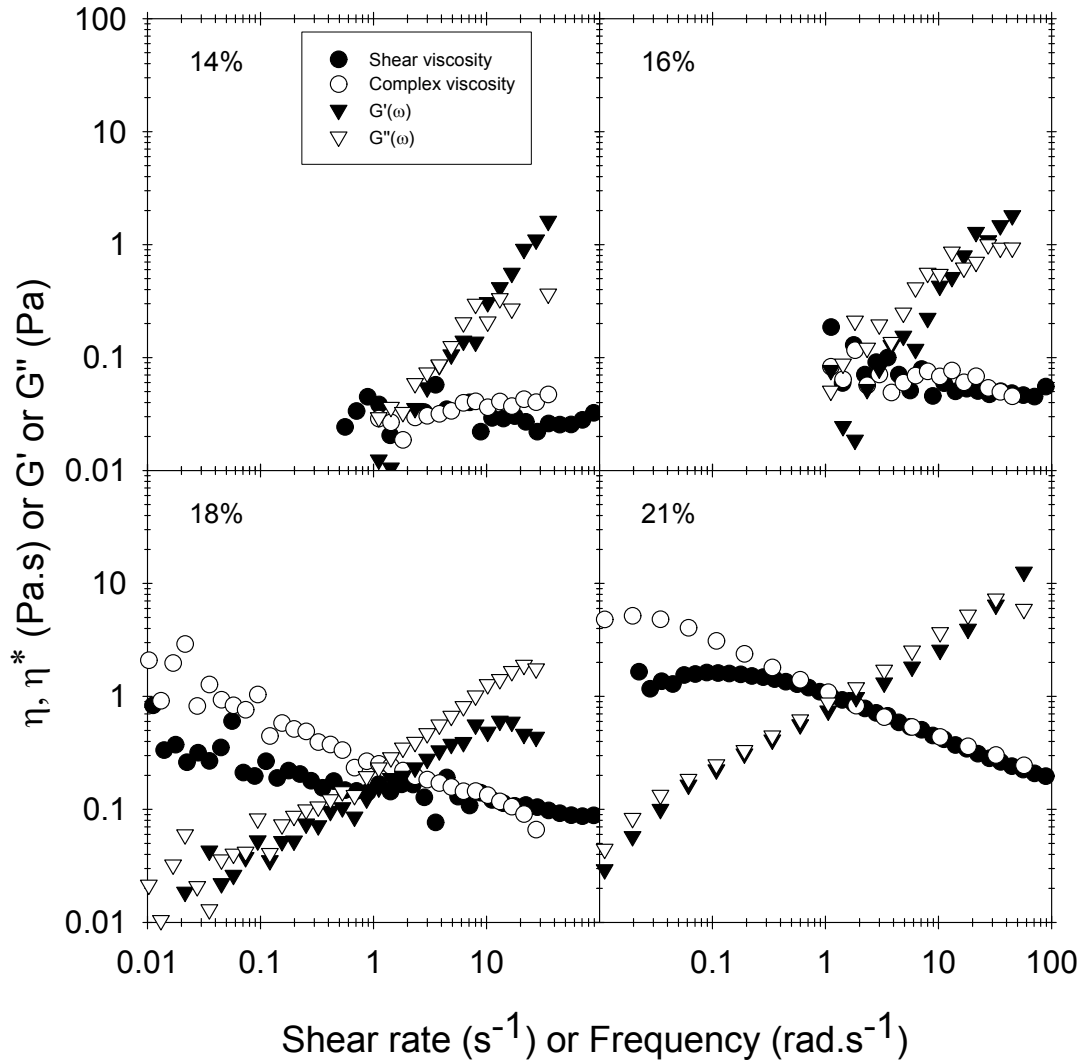
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763 **Figure 3**

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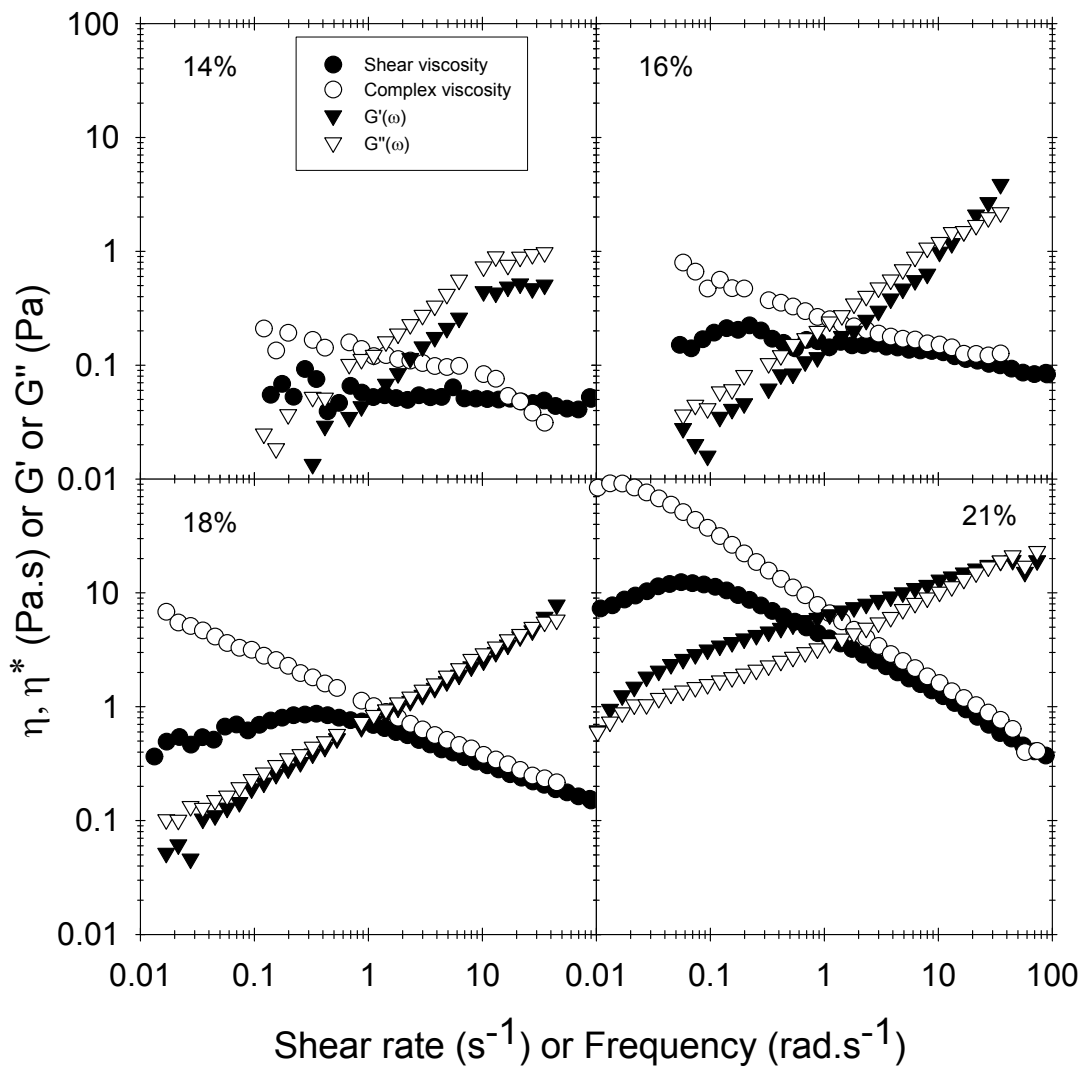
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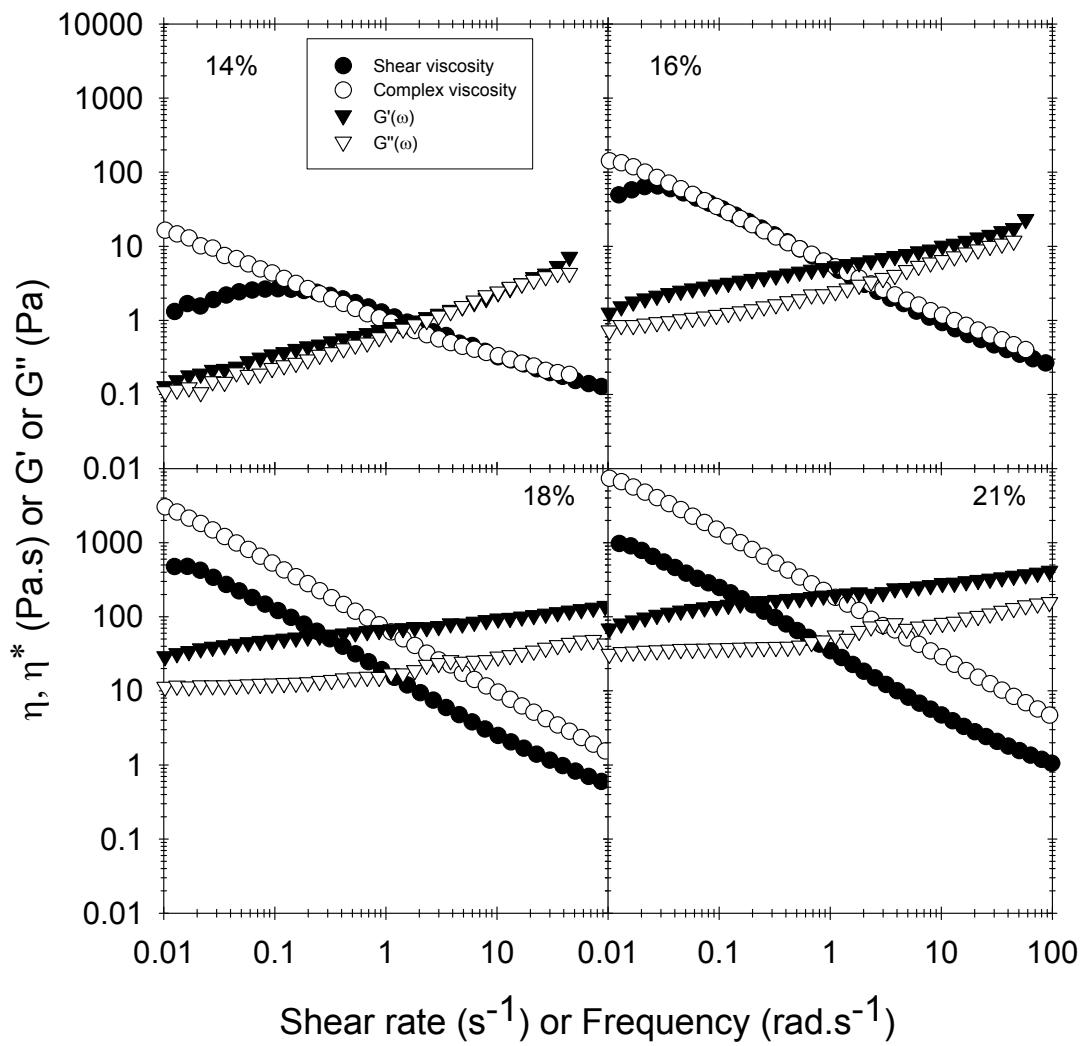
768 **Figure 4**



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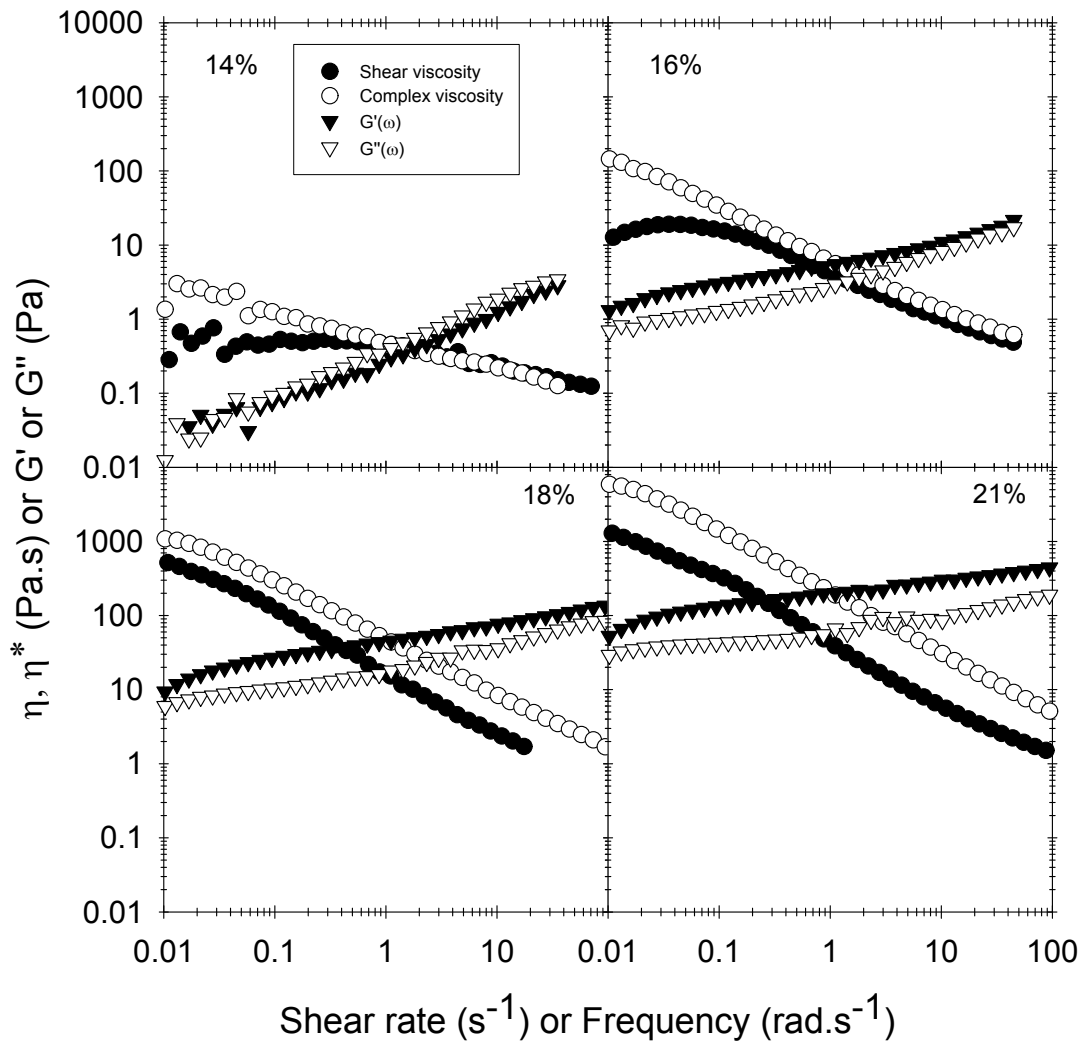
771 **Figure 5**



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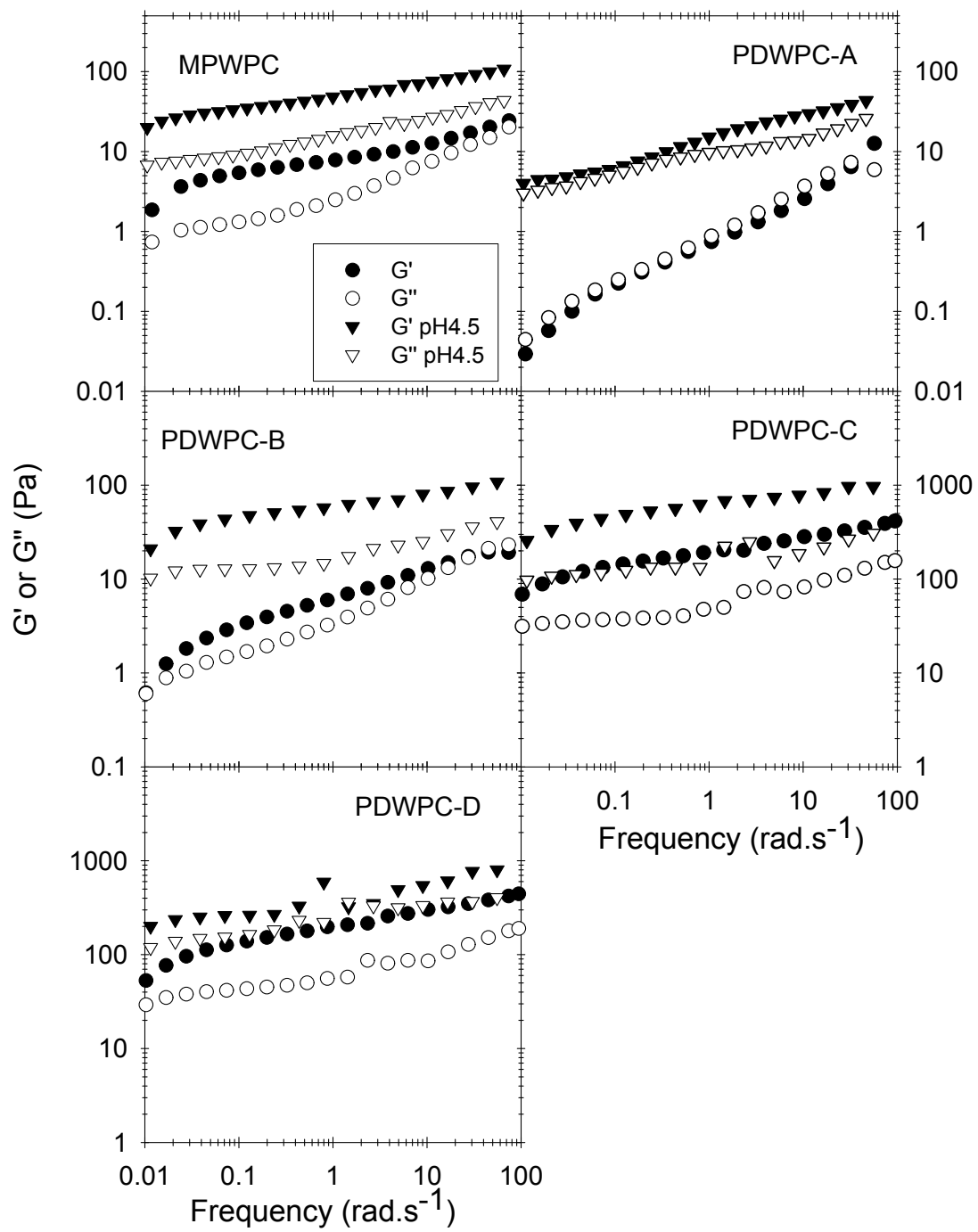
775 **Figure 6**



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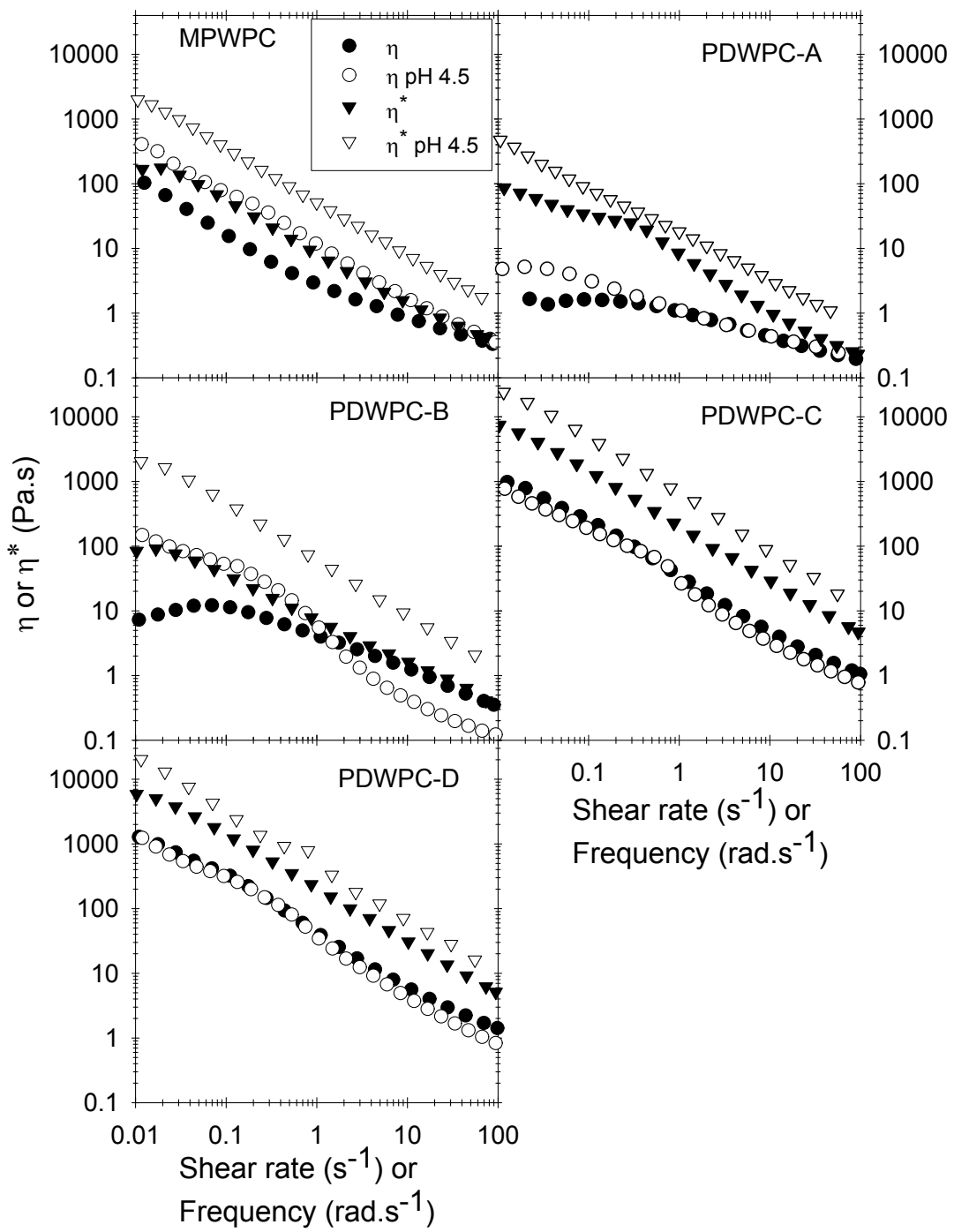
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778 **Figure 7**



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780 **Figure 8**



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782 **Figure 9**

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