

New insights into the mechanism of rehydration of milk protein concentrate powders determined by Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS)

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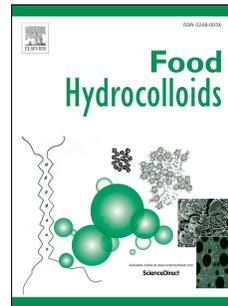
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New insights into the mechanism of rehydration of milk protein concentrate powders determined by Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS)

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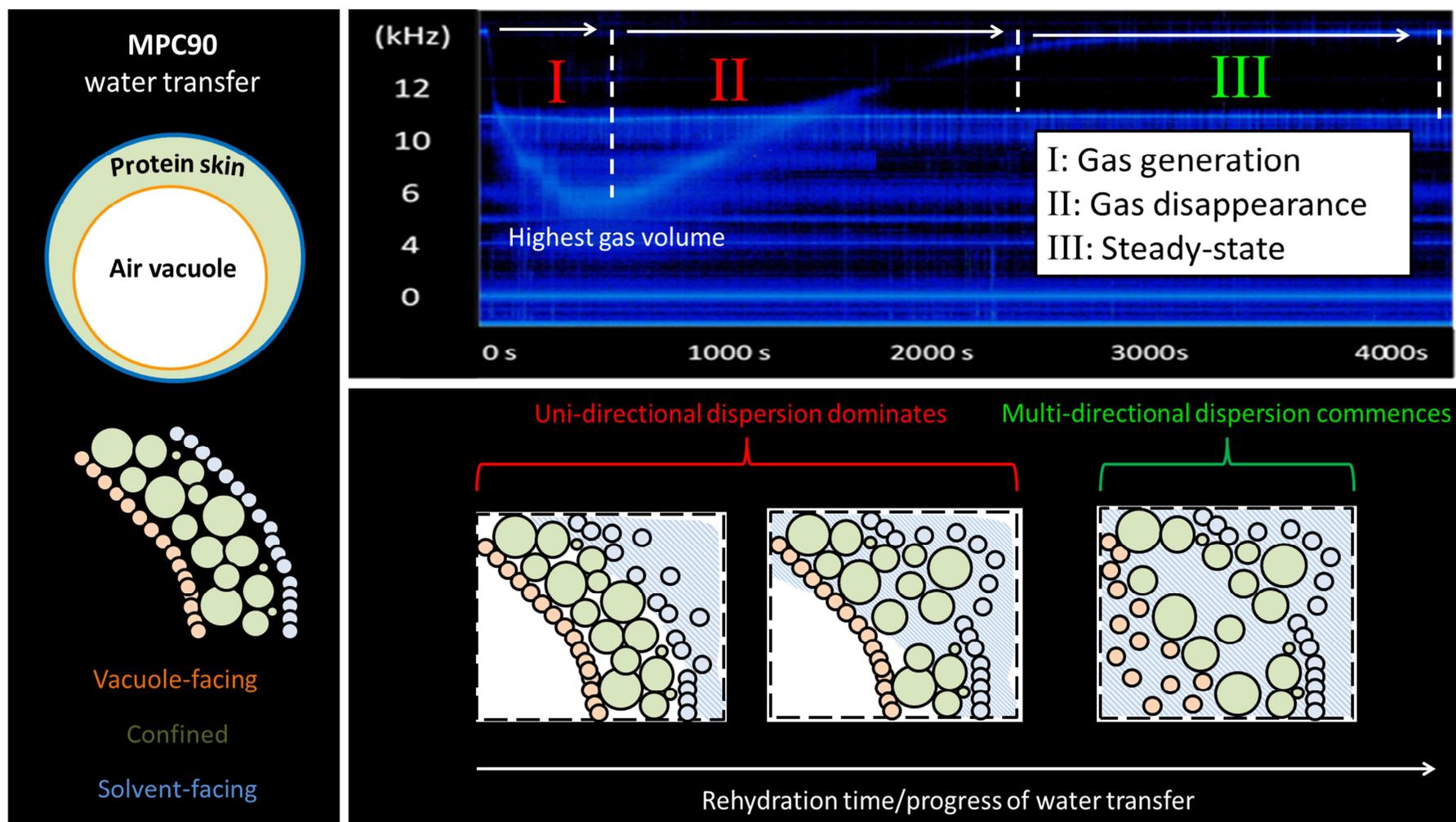


Figure 10.

1 **New insights into the mechanism of rehydration of milk protein**
2 **concentrate powders determined by Broadband Acoustic**
3 **Resonance Dissolution Spectroscopy (BARDS)**

4

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16

17 **Abstract**

18 This study investigated the transfer of water into milk protein concentrate (MPC) powder
19 particles using Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS) as a
20 detection method for the first time. BARDS analysis is based on an acoustic phenomenon
21 which occurs during powder rehydration. Release of air from the powder into the solvent
22 during rehydration leads to outgassing in the solvent, which results in changes in solvent
23 compressibility that are monitored through accompanying changes in induced resonance
24 frequencies in the dissolution vessel. BARDS confirmed that water transfer into MPC
25 particles became increasingly inhibited as protein content of the powder increased. The
26 reproducibility of the data indicates that air release from internal vacuoles within powder
27 particles in high-protein MPCs is a highly ordered process, occurring over a protracted time
28 scale. Kinetic modelling of gas volume data from BARDS confirmed that the release of
29 occluded air caused the changes in solvent compressibility during rehydration. The
30 physicochemical properties of solubilised protein had a slight inhibitory effect on escape of
31 bubbles from the solvent, but the primary factor limiting gas release from high-protein MPCs
32 was water transfer into powder particles and the concomitant release of occluded air into the
33 solvent. In agreement with many previous studies, cryo-SEM analysis showed that particles
34 in high-protein MPCs were slow to disperse; the current study, in addition, highlights
35 inhibited water transfer into particles as another factor which may contribute to their poor
36 rehydration properties. A potential link between inhibited water transfer and poor
37 dispersibility is proposed.

38

39

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43 **Keywords:** MPC, BARDS, rehydration, solubility, water transfer, particle structure

44 1. Introduction

45 Milk protein concentrate (MPC) powders are recently developed ingredients which
46 contain the two major protein fractions of bovine milk at the ratio that they occur naturally in
47 milk (80:20 casein:whey protein). MPCs are typically manufactured using pressure-driven
48 membrane separation processes, where ultrafiltration (UF) alone, or a combined UF and
49 diafiltration (DF) process, is used to concentrate protein while removing smaller molecules
50 including lactose, salts, non-protein nitrogen (Carr and Golding, 2016). After membrane
51 processing, MPCs are usually spray-dried into powders. Currently, the most widely used
52 MPC powders in commercial applications are the high-protein varieties (i.e., those containing
53 $\geq 80\%$ protein). High-protein MPCs are exploited for their functional attributes (e.g.,
54 viscosity, emulsification, curd-forming ability) and nutritional features (e.g., high protein,
55 low lactose) in a range of commercial applications (Agarwal *et al.*, 2015).

56 When milk protein concentrate (MPC) powders are manufactured to a final protein
57 content $\geq 70\%$, solubility is commonly impaired (Crowley *et al.*, 2015), with the rate of
58 liberation of casein micelles from powder particles during rehydration reducing with
59 increasing protein content of the powders (Mimouni *et al.*, 2010b). As casein is the
60 predominant component in high-protein MPCs, and the majority of caseins exist in the
61 micellar state, the persistence of these poorly-dispersible particles for extended periods after
62 wetting and submersion of the powder can result in suspensions with an unacceptably high
63 quantity of sedimentable solids (Havea, 2006; Sikand *et al.*, 2011). Furthermore, after
64 extended rehydration, these particles may not be of sufficient density to sediment, but may
65 still remain suspended as large, highly-hydrated particles (Fang *et al.*, 2011; Crowley *et al.*,
66 2015). Incomplete rehydration of MPCs is an issue which is encountered both during mixing
67 of dried ingredients by processors and reconstitution of dried products by consumers, and can
68 have a negative influence on the functional and sensory properties of the final product (Carr
69 and Golding, 2016).

70 Micellar casein is primarily responsible for the solubility issues encountered during
71 the rehydration of MPCs (McKenna, 2000; Anema, 2006; Havea, 2006; Mimouni *et al.*,
72 2010a; Gazi and Huppertz, 2014). The particular solubility issues associated with MPCs are
73 not found in whey protein-dominant powders (i.e., whey protein concentrates/isolates),
74 casein-dominant powders which are relatively low in protein (i.e, skim milk/nonfat dry milk
75 powders) or high-protein casein-dominant powders which do not contain micellar casein
76 (e.g., sodium caseinate). Micellar casein concentrates (MCCs), powders with higher

77 casein:whey protein ratios than MPCs, are known to have similarly poor, and even more
78 challenging, rehydration performance (Crowley *et al.*, 2016); when levels of lactose or whey
79 proteins are increased in MCCs they dissolve more quickly, due to a concurrent decrease in
80 the level of micellar casein and possible improvements in the water transfer properties of the
81 powder due to the presence of the more soluble components (Richard *et al.*, 2012).

82 In high-protein MPC powders, casein micelles are considered to be the molecular
83 building blocks of a 'skin' at the surface of primary powder particles, which may prevent the
84 release of casein micelles during rehydration (McKenna, 2000; Mimouni *et al.*, 2010b; Fyfe
85 *et al.*, 2011; Crowley *et al.*, 2016; Ji *et al.*, 2016). The solubility of MPCs deteriorates further
86 during storage under adverse conditions (Anema *et al.*, 2006; Fyfe *et al.*, 2011; Gazi and
87 Huppertz, 2014), due to the decreased solubility of micellar casein, while the solubility of
88 whey proteins is largely retained unless they have been denatured to a significant degree
89 during processing (Gazi and Huppertz, 2014). The central role of micellar casein in the
90 development of insolubility issues in MPCs is further supported by some of the techniques
91 which have been used to improve their solubility, including high pressure treatment (Udabage
92 *et al.*, 2012), ion-exchange (Bhaskar *et al.*, 2001) and CO₂ injection (Marella *et al.*, 2015), all
93 of which are primarily based on structural modification of casein micelles (Carr and Golding,
94 2016).

95 There is a need to develop *in-situ* techniques for the dynamic monitoring of powder
96 rehydration phenomena, as this will allow the identification of the stages (i.e., wetting, water
97 transfer, or dispersion) which are responsible for prolonged rehydration times (Fang *et al.*,
98 2008; Crowley *et al.*, 2016). Dynamic studies of MPC powder rehydration have primarily
99 focused on advanced stages of rehydration, such as dispersion, which has been identified as
100 the rate-limiting step in the rehydration process for MPCs in experiments where the changes
101 in particle size over time were measured (Mimouni *et al.*, 2009; Fang *et al.*, 2011). However,
102 other than the study of Hauser and Amamcharla (2016) on commercial MPC80, there are
103 limited studies available in the literature in which water transfer into particles during the
104 rehydration of different MPC powders has been investigated. Water transfer has been studied
105 in MCCs by Schuck *et al.* (2002) and Richard *et al.* (2012) using nuclear magnetic resonance
106 relaxometry and ultrasound attenuation measurements, respectively, with both studies
107 demonstrating that water transfer can be markedly inhibited in MCCs. Bouvier *et al.* (2013)
108 demonstrated that increasing the size and number of pores in particles can improve the
109 rehydration properties of MCC powders, supporting the concept that enhancing water transfer
110 can improve the dispersibility of these powders.

111 Generating data on water transfer phenomena in MPCs could potentially inform
112 strategies to modify particle structure (i.e., during or after spray drying) in order to improve
113 their rehydration characteristics. Thus, in this study, a new form of acoustic spectroscopy,
114 Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS), was used to monitor
115 water transfer related phenomena in a range of MPC powders for the first time. BARDS is an
116 analytical platform technology with multiple applications, such as blend uniformity analysis,
117 discrimination of polymorphs and drug loading on sugar spheres for controlled release
118 formulations (Fitzpatrick *et al.*, 2012a, 2012b, 2014).). The technique is based on real-time
119 changes in the compressibility of a solvent as a solute dissolves, which can be monitored
120 acoustically via changes in induced resonant frequencies of the dissolution vessel. In this
121 study, the rehydration behaviour of MPCs with varying protein contents was assessed over
122 time using BARDS. Experiments were devised to isolate the influence of water transfer on
123 changes in gas volume during rehydration. A novel kinetic approach was used to confirm the
124 role of occluded air release in determining BARDS spectra. The influence of serum-phase
125 composition (i.e., soluble protein) on the escape of gas from the solvent was considered for
126 the first time in a study of dairy powder rehydration based on changes in gas volume. Cryo-
127 SEM micrographs were also collected during the rehydration of selected powders to establish
128 a potential link between water transfer and particle dispersion-state.

129

130 **2. Experimental**

131 *2.1. Materials*

132 Crowley *et al.* (2014a, b) described the manufacturing protocol for pilot-scale
133 production of the MPC powders used in the current study. In brief, pasteurised skim milk was
134 subject to UF (MPC50, MPC60) or UF and DF (MPC70, MPC80, MPC85, MPC90) to
135 different protein concentration factors at 50°C with 10 kDa molecular weight cut-off
136 membranes. MPC35 did not undergo any membrane filtration, and is essentially skim milk
137 powder. MPC35, MPC50, MPC60 and MPC70 were evaporated before being spray-dried,
138 while MPC80, MPC85 and MPC90 were not subjected to evaporation. Spray drying involved
139 nozzle atomisation, an air inlet temperature of 185-190°C and an outlet temperature of 85-
140 90°C. The composition and selected physical properties (measured as described by Crowley
141 *et al.* (2014a, b) of the MPC powders is provided in Table 1. Analar grade KCl was
142 purchased from Sigma Aldrich. The solvent used for rehydration experiments was deionised
143 water unless otherwise indicated.

144

145 **[Table 1 about here]**

146

147 2.2. Instrumentation

148 The BARDS spectrometer consists of a closed chamber with a dissolution vessel
149 (soda lime glass), microphone (Sony ECM-CS10, range 100 Hz – 16 kHz), a magnetic stirrer
150 and follower. A schematic diagram is shown in Figure 1, demonstrating the principle of
151 BARDS as applied in the current study and the basic components of the apparatus. There is
152 access at the front of the chamber for the dissolution vessel and at the top in order to place a
153 sample in a weighing boat on an automated tipper motor for introduction of the powder. The
154 microphone is positioned above the top of the glass within the housing for these studies. The
155 glass, containing 25 mL of deionised water, is placed on the stirrer plate. The stirrer motor
156 underneath is positioned so as to allow the magnetic follower to gently tap the inner vessel
157 wall. In this way, the follower acts as a source of broadband acoustic excitation, thereby
158 inducing various acoustic resonances in the glass, the liquid and the air column above the
159 liquid. The audio is sampled at a rate of 44.1 kHz. A fast Fourier transform is applied to the
160 signal, resulting in a typical BARDS frequency response. The resonances of the liquid vessel
161 are recorded in a frequency band of 0-20 kHz. The frequency response was measured during
162 the rehydration of 0.04-0.20% (w/v) suspensions of MPCs in 25 mL water.

163

164 **[Figure 1 about here]**

165

166 2.2.1 Theoretical Background of BARDS

167 The BARDS response results from changes in the compressibility of a solvent during
168 the dissolution of a compound, in which compressible gas bubbles are introduced or
169 generated. Changes in compressibility alter the speed of sound resulting in frequency changes
170 of induced acoustic resonances within the solvent. The principles underlying the BARDS
171 response are as follows. The sound velocity (v) in a medium (m s^{-1}), whether air or liquid
172 phase, is determined by Equation 1.

173

$$174 \quad v_{(\text{sound})} = \sqrt{\frac{1}{K \cdot \rho}} \quad \text{Equation (1)}$$

175

176 Where ρ is the density (kg m^{-3}) and K is the compressibility (inverse of the bulk modulus) of
 177 the medium (Pa^{-1}). Generation of micro bubbles in a liquid decreases the density in a
 178 negligible way in comparison to a large increase in compressibility. The net effect is a
 179 significant reduction of the sound velocity in the liquid. The following relationship between
 180 the fractional bubble volume and the sound velocity in water was derived by Frank S.
 181 Crawford, as given in equation 2 (Crawford, 1982):

$$\frac{v_w}{v} = \sqrt{(1 + 1.49 \times 10^4 \cdot f_a)} \quad \text{Equation (2)}$$

182
 183
 184 where v_w and v are the velocities of sound (m s^{-1}) in pure and bubble-filled water,
 185 respectively, and f_a is the fractional volume occupied by air bubbles. Equation 2 is based on
 186 an approximation presented originally by Wood (1930).
 187

188 BARDS analysis of an induced acoustic excitation of the vessel containing the fluid is
 189 focused on the lowest variable frequency time-course, i.e., the fundamental resonance mode
 190 of the liquid. The fundamental resonant frequency is determined by the sound velocity in the
 191 liquid and the approximate but fixed height of the liquid level, which corresponds to one
 192 quarter of its wavelength. The frequency response is described as:

$$freq = \frac{freq_w}{\sqrt{1 + 1.49 \times 10^4 \cdot f_a}} \quad \text{Equation (3)}$$

193
 194
 195 where $freq_w$ and $freq$ are the resonance frequencies (kHz) of the fundamental resonance
 196 modes in pure and bubble-filled water, respectively. A comprehensive outline of the
 197 principles and underlying processes involved in BARDS analysis is given by Fitzpatrick *et al*
 198 (2012a).
 199

200 2.3. Experimental procedure for BARDS experiments

201 In a typical experiment, the spectrometer records the steady-state resonances of the
 202 system as a reference for 30 s after the stirrer has been set in motion (Figure 1, panel 1). The
 203 pitch of the resonance modes in the solution change significantly when the powder is added
 204 (Figure 1, panel 2), before gradually returning to steady-state over several minutes (Figure 1,
 205 spectrum in panel 3). The amounts used are expressed as solid/liquid concentration (w/v) in
 206 all figures and throughout the text. Gas oversaturation of water prior to introduction of
 207 powders was removed through agitation by shaking vigorously for 60 s and then resting for
 208

209 10 min. Otherwise, remaining gas oversaturation may lead to an over-response (Fitzpatrick *et*
210 *al.* 2013).

211 The frequency-time response of the fundamental resonance is presented as manually
212 extracted data from the total acoustic response. The steady-state frequency before addition of
213 the powder is designated as the 'volume line', so called as it varies depending on the liquid
214 volume in the vessel. Spectra were recorded for 3000-4000 s depending on the rate of return
215 of the BARDS response to steady state. All experiments were performed in duplicate at
216 ambient temperature (~22°C) and atmospheric pressure. Average readings with error bars
217 representing the standard deviation are presented.

218

219 *2.4. Characterisation of the microstructure of MPC powders in their dry state*

220 Scanning electron microscopy (SEM; Philips XL30 FEG ESEM) was used to
221 characterise the microstructure of MPC35, MPC70 and MPC90 to assess any morphological
222 differences. MPC powder samples were placed upon double-sided adhesive conductive
223 carbon tape, sputter-coated with gold and scanned at 10 kV.

224

225 *2.5. Characterisation of the microstructure of MPC powders during rehydration*

226 Cryogenic scanning electron microscopy (Cryo-SEM; Philips XL30 FEG ESEM), with
227 a Gatan low temperature preparation system, was used to visualise the microstructure of
228 MPC35 and MPC90 at rehydration times of 100, 1000 and 3000 s. Cryo-SEM analysis was
229 performed to assess differences in particle dispersion between the two powders, to supplement
230 water transfer data generated using BARDS. One drop of liquid was frozen to approximately -
231 180 °C in liquid nitrogen slush. Samples were then fractured and etched for 1 min at a
232 temperature of -95 °C inside the preparation chamber. Afterwards, samples were sputter coated
233 with gold and scanned at 3 kV, during which the temperature was maintained below -160 °C by
234 addition of liquid nitrogen to the system.

235

236 **3. Results**

237 *3.1 Composition and physical properties of the MPC powders*

238 Data related to the composition and physical properties of the MPCs are shown in
239 Table 1. Reductions in lactose and mineral levels were measured in the MPC powders,
240 corresponding with increasing protein concentration. Particle size increased with increasing
241 protein content for MPC35, MPC50 and MPC60, and decreased thereafter as protein content
242 increased further. There were no apparent trends in the volume of interstitial and occluded air
243 when MPC35, MPC50, MPC60 and MPC70 were compared; however, MPC80, MPC85 and
244 MPC90 were 2-3 times more aerated than the former powders, although there were only
245 minor differences within this class of high-protein MPC powders.

246

247 3.2 Interpretation of BARDS profiles

248 Figure 1, panel 3, shows a typical BARDS spectrum during the rehydration of
249 MPC90. The acoustic frequency profile of interest is called the fundamental curve. The
250 frequency minimum (f_{min}) represents an equilibrium between the rate of introduction of gas as
251 bubbles into solution and the rate of elimination of these bubbles at the surface of the
252 solution. In BARDS analysis, the fundamental curve is used to make comparisons between
253 individual experiments. The acoustic frequencies of the vessel remained steady for the first
254 30 s until the addition of MPC90; thereafter, the resonant frequency at 14 kHz decreased to 6
255 kHz and gradually returned to steady-state. The constant frequency at 11 kHz is just one of
256 many resonant frequencies of the vessel that is not dependent on the liquid compressibility
257 and therefore remained unchanged as gas volume levels fluctuated.

258 Figures 2 and 3 show the acoustic profiles for all seven MPCs with a concentration of
259 0.2%. There is a gradual increase in the deflection to f_{min} with increasing protein content.
260 Powders with higher protein concentrations exhibited a distinct change in the rate of gas
261 release into the solvent (reduced down-slope) compared to lower protein powders, as
262 indicated by the increased amount of time required to reach f_{min} . The disappearance of gas
263 from the solvent after f_{min} also proceeded more slowly (reduced up-slope) as protein content
264 of the MPC powder increased, which resulted in considerably extended times to reach steady-
265 state. Most notable was the time required (~3000 s) to reach steady-state for MPC90.

266

267 **[Figure 2 about here]**

268 The acoustic profiles (Figure 2) strongly indicate differences in the volume of gas generated,
269 the rate of gas release from the powders, and the rate of gas disappearance from the solvent
270 during the rehydration of different MPCs. These factors were investigated more closely by
271 tracking changes in gas volume in the following sections.

272

273 *3.3 Changes in compressible gas volume during rehydration of MPC powders*

274 Equation 3 was applied to the BARDS frequency data from the MPC experiments
275 (Figure 2) to generate data relating to the fractional gas volume (f_a) occupied by compressible
276 gas during the dissolution of 0.2% of each of the MPCs. The gas volume plots presented in
277 Figures 3 (A) and (B) concern absolute volumes ($f_a \times V_{solution}$). The initial up-slope indicates
278 the rate at which gas was released from the powder. The data suggests that a significant
279 change in rehydration behaviour occurred when the protein content exceeded 80% in the
280 MPC powders. MPC35 generated a negligible gas volume during rehydration (see higher
281 resolution data in Figure 3, B). MPC50 and MPC60 exhibited a very rapid release of a limited
282 quantity of gas, the disappearance of which from the solvent began immediately and
283 proceeded rapidly. Conversely, for MPC80, MPC85 and MPC90, there was a very gradual
284 increase in the gas volume to a high maximum, after which point gas remained constant in
285 the system for ~ 200 s, due to a balance of gas release and disappearance, before gas
286 disappearance from the liquid surface became dominant. The steady increase in the
287 compressibility of the solvent for these powders during ~ 500 s of rehydration indicates that
288 the immersed particles themselves, containing occluded air prior to significant water transfer,
289 are non-compressible and that, as such, the release of gas from the particles contributes to
290 changes in the compressibility of the solution. If the particles themselves were compressible,
291 an immediate and marked increase in gas volume would be expected to occur as soon as the
292 powder submerged.

293

294 **[Figure 3 about here]**

295

296

297 *3.4 Kinetic analysis of changes in compressible gas volume during rehydration of the MPCs*

298 When the gas volume data (Fig. 3 A, B) is plotted using a logarithmic scale, as shown
299 in Figure 3 (C), the disappearance rate constant (k) for compressible gas in the solution is
300 given by the descending slope (assuming a first-order process). Table 2 presents the results of
301 this gas disappearance analysis, with values for k and the time range from which the
302 descending slope was calculated. For the MPC suspensions at the highest concentration
303 studied (0.2%), a gradual decrease in gas disappearance rate is observed with increasing
304 protein content of the powder. The k value of the lowest protein powder (MPC35) was five
305 times that of the highest protein powder (MPC90), indicating a profound shift in water
306 transfer behaviour.

307

308 **[Table 2 about here]**

309

310 Based on visual assessment of wetting behaviour, kinetic data in Table 2 and gas volume-
311 time plots (Fig. 3), it is possible to distinguish four categories of MPC based on data for 0.2%
312 systems:

313

314 **Fast wetting/fast water transfer/fast gas disappearance: MPC35 and MPC50**

315 These powders wetted rapidly at the water surface and underwent fast sinking. The volume
316 response-time curves for these samples show a subsequent fast release of compressible gas
317 from the powder into the solvent, indicating that water transfer into particles was rapid after
318 sinking. In contrast to the MPC50, the MPC35 response seems to indicate a relatively slower
319 rate of water transfer, despite having a lower protein content. Both powders exhibited high
320 gas disappearance rates ($k \approx 1-3 \times 10^{-2} \text{ s}^{-1}$) compared to the other MPCs. These results are
321 generally in line with previous studies demonstrating that low-protein MPCs have good
322 solubility characteristics (Crowley *et al.*, 2015; Sikand *et al.*, 2011)

323

324 Fast wetting/fast water transfer/intermediate gas disappearance: MPC60 and MPC70

325 These samples exhibited rapid wetting at the powder surface and gas generation in the
326 solvent, suggesting that water transfer into particles in these powders was not severely
327 inhibited compared to MPC35 and MPC50. Like MPC50, these powders exhibited a more
328 rapid rate of water transfer than MPC35. However, both powders exhibit slower gas
329 disappearance rates ($k \approx 3.7\text{-}6.6 \times 10^{-3} \text{ s}^{-1}$) than MPC35 and MPC50, indicating that escape
330 of bubbles from the solvent was inhibited compared to the lower protein powders, likely due
331 to the increasing influence of solubilised protein. For example, a 0.2% solution of MPC70
332 will have approximately double the protein content of MPC35. Particles in this set of MPCs
333 with <80% protein disperse relatively quickly (Crowley *et al.*, 2015), and therefore increasing
334 the protein content from MPC35/MPC50 to MPC60/MPC70 may have increased the levels of
335 soluble protein to a degree sufficient to inhibit bubble escape (Ybert and di Meaglio, 1998).

336

337 Slow wetting/slow water transfer/intermediate gas disappearance: MPC80 and MPC85

338 The initial part of the response was likely influenced by slow wetting, with MPC80 and
339 MPC85 observed to require ~200 s to fully disappear from the liquid surface; however, this
340 cannot account for the 500 s of gas generation which elapsed prior to the initiation of the gas
341 disappearance phase, which was strongly indicative of inhibited water transfer into powder
342 particles. As with MPC60 and MPC70, intermediate gas disappearance rates ($k \approx 3.4 - 3.9 \times$
343 10^{-3} s^{-1}) were measured, which suggests that the period of inhibited water transfer did not
344 continue into the gas disappearance phase and influence derived k values. Thus, the effect of
345 soluble proteins might be considered to dominate gas disappearance, as per the lower protein
346 MPCs.

347

348 Slow wetting/slow water transfer/slow gas disappearance: MPC90

349 Similarly to MPC80 and MPC85, slow wetting was observed for MPC90 (~200 s). The initial
350 part of the response of MPC90 is also similar to that of MPC80 and MPC85, and strongly

351 indicates inhibited water transfer into the submerged particles. The gas disappearance rate for
352 MPC90 was the lowest of all the powders ($k \approx 1.9 \times 10^{-3} \text{ s}^{-1}$), and suggested that water
353 transfer may have continued during the gas disappearance phase.

354

355 The slow gas generation for high-protein MPCs strongly indicates inhibition of water transfer
356 into powder particles. However, it is not clear, especially for MPC90, which of the two
357 primary phenomena (water transfer into particles and gas elimination from the solvent) are
358 rate-determining for the observed trends in gas disappearance based on the kinetic data for
359 0.2% systems alone. Further analysis of the concentration-dependency of the BARDS
360 response for different MPCs was performed to obtain more reliable mechanistic and kinetic
361 information.

362 The concentration-dependency of the BARDS response for four of the seven MPCs (MPC35,
363 MPC70, MPC80 and MPC90), spanning the four aforementioned categories, is shown in
364 Figure 4. The comparative kinetic analysis of the BARDS data is based on the related gas
365 volume data and is presented in Figure 5 using a logarithmic scale.

366 **[Figure 4 about here]**

367

[Figure 5 about here]

The results for individual powders can be summarised as follows:

368 **MPC35** (Figure 5, A):

369 An immediate, very rapid gas disappearance was observed for 0.04 and 0.08% systems ($k \approx 5$
370 $\times 10^{-2} \text{ s}^{-1}$). A short time period (~200 s) of constant gas volume was observed at the higher
371 concentrations of 0.12% (~100 s) and 0.16 and 0.20% (~200 s) before the gas volume started
372 to decrease. The periods of constant volume may be attributed to slower powder wetting and
373 uptake into the solvent, which was observed with higher quantities of added MPC35. In the

374 gas disappearance phase, a k value of $\sim 1 \times 10^{-2} \text{ s}^{-1}$ was calculated for concentrations of 0.08
375 - 0.20%.

376 **MPC70** (Figure 5, B):

377 The gas disappearance rate for the concentrations 0.04 – 0.16% decreased gradually with
378 time. Therefore, the curves have been characterized by two gas disappearance rate constants,
379 an initial fast release and a subsequent slow release: the values are $k \approx 1.2\text{-}1.7 \times 10^{-2} \text{ s}^{-1}$ for
380 the initial part of each curve and $k \approx 7\text{-}8 \times 10^{-3} \text{ s}^{-1}$ for the terminal parts. The gas
381 disappearance for the 0.2% system was much slower than these lower concentrations with $k \approx$
382 $6.6 \times 10^{-3} \text{ s}^{-1}$ for the initial part and $k \approx 3.7 \times 10^{-3} \text{ s}^{-1}$ for the terminal part of the curve. The
383 overall decrease in disappearance rate with rehydration time and with increase of MPC70
384 concentration, may suggest an increasing influence of solvent properties (e.g., increasing
385 viscosity or ‘protein drag force’ effects) on the release of bubbles from the solvent as protein
386 is solubilised.

387

388 **MPC80** (Figure 5, C):

389 The time taken for the gas volume to reach its maximum value increased with increasing
390 concentration, due to the influence of increasingly longer wetting times (200 s at the highest
391 mass added). The gas disappearance rate constants decreased with increasing concentration
392 from $k \approx 6.7 \times 10^{-3} \text{ s}^{-1}$ at 0.04% to $k \approx 3.4 \times 10^{-3} \text{ s}^{-1}$ at 0.2%.

393

394 **MPC90** (Figure 5, D):

395 Compared to MPC80, the concentration-dependency of the time for the gas volume to reach
396 its maximum seemed less prominent with MPC90, despite this powder having similar wetting
397 times. In addition, the gas disappearance rate appears to be relatively independent of
398 concentration ($k \approx 2.0\text{-}3.0 \times 10^{-3} \text{ s}^{-1}$) compared to the other MPCs.

399

400 The rate-limiting stage for the descending slope (representing gas disappearance) seems to be
401 gas elimination from the solvent MPC70. Values for k decrease with increasing
402 concentration, indicating that increasing levels of solubilised components may have retarded
403 bubble escape to a greater degree. On the other hand, for MPC90, gas release from MPC
404 particles appears to be rate-limiting, as gas disappearance was effectively independent of
405 concentration. A slow and ordered process of water transfer into MPC90 particles would
406 explain this observation.

407

408 The MPC80 data indicate a concentration-dependent transition between the processes
409 described which determine the gas disappearance rate for MPC70 and MPC90. Like MPC90,
410 there is evidence that this powder has poor water transfer properties, due to the extended
411 duration of its gas generation phase, but its gas disappearance behaviour is broadly similar to
412 MPC70. It is proposed that the factor which extends the gas disappearance phase of MPC90
413 compared to MPC80 is a slower water transfer process.

414

415 *3.6 Validation of water transfer as key stage influencing BARDS spectra for MPC90*

416 The data presented in previous sections indicate that inhibited water transfer into particles in
417 high-protein MPCs strongly influences the BARDS spectra, with powders such as MPC90
418 containing particles which require longer water transfer times. An experiment was designed
419 to investigate whether the slow decrease in the compressible gas volume for high-protein
420 MPC samples (especially MPC90) is due to steady transfer of gas out of the MPC particles
421 (during water transfer) or due to other processes which affect the loss of gas at the liquid
422 surface - for instance, an increase in viscosity, surface tension or drag forces acting on
423 ascending bubbles (Ybert and di Meaglio, 1998). To this end, KCl was used as a monitoring
424 compound, to investigate whether the physicochemical properties of rehydrated MPC90 (post
425 steady-state) inhibited the ability of gas to escape from the liquid. Figure 6 (A) shows the
426 BARDS responses during the dissolution of 0.5 M KCl in water and also the dissolution of
427 0.2% MPC90 in water. KCl exhibits immediate release of gas and a fast return to steady state
428 within 200 s.

429

430 [Figure 6 about here]

431

432 A second experiment was performed whereby the same amount of KCl was added to a
433 solution of 0.2% MPC90 which had been rehydrated until a steady-state BARDS response
434 was achieved (Fig. 6, B). Again, there was an immediate generation of gas observed, but the
435 return to steady-state took ~10 times longer due to replacement of water with MPC90
436 solution. This result shows that the presence of soluble proteins impedes gas disappearance.
437 Despite the slower escape of gas from the liquid, it took a significantly shorter time for gas
438 from KCl to disappear from MPC solution compared to the disappearance of gas during the
439 rehydration of MPC90. The first-order k values can be derived from the descending slopes in
440 Figure 6 (B), and were found to be $2.8 \times 10^{-3} \text{ s}^{-1}$ for KCl dissolved in MPC90 solution
441 compared to the lower k value of $1.5 \times 10^{-3} \text{ s}^{-1}$ for MPC90 on its own. This strongly indicates
442 that for MPC90 the terminal gas disappearance rate is determined by the process of continued
443 water transfer into particles generates compressible gas from MPC90 during rehydration.

444 In pharmacokinetics, an analogous process to that observed for MPC90 rehydration can be
445 described in which the terminal stage of the concentration time-course of a drug in the blood
446 reflects the drug absorption process instead of the elimination process as a 'flip-flop' system
447 (Boxenbaum, 1998). In this study, generation of compressible gas bubbles through water
448 transfer into particles can replace absorption, in the pharmacokinetic sense, for the kinetic
449 analysis of MPC90 rehydration presented in the following section.

450

451 *3.7 Verification that occluded air accounted for total gas volume using flip-flop kinetics*

452 The gas volume time-course of MPC90 (see Figure 3, A) was used to establish the total
453 amount of compressible gas that was produced during the rehydration experiment. An
454 approach was followed similar to that used in pharmacokinetic studies, in which one
455 distinguishes the absorption of a drug into the body, its distribution and its subsequent
456 elimination. The concentration-time profile is determined in the central compartment
457 (blood/plasma). The area under the concentration/time curve (AUC), combined with the drug

458 distribution volume (V_d) and its first-order elimination rate constant (k_{el} , s^{-1}) are used to
459 calculate the dose (D) that has entered the central compartment using equation 4.

$$460 \quad D = AUC \times V_d \times k_{el} \quad \text{Equation (4)}$$

461 In an adjusted approach used for the gas volume analysis, the absorption is replaced by the
462 generation of compressible gas into the solution (the central compartment) following addition
463 of MPC90 to the solvent. The dose administered in pharmacokinetics becomes the total
464 amount of compressible gas produced. In contrast to pharmacokinetics, the distribution
465 volume (V_d) is now simply the volume of the solution ($V_{solution}$) (Rowland and Tozer, 1989).
466 The total amount of compressible gas produced during dissolution (D_{gas}) can then be
467 calculated using Equation 5.

$$468 \quad D_{gas} = AUV \times k_{el} \quad \text{Equation (5)}$$

469 Where D_{gas} (mL) is the total amount of compressible gas produced during the rehydration of
470 MPC90. AUV (mL.s) is the total area under the gas volume/time curve (the volume of
471 compressible gas is calculated as $f_a \times V_{solution}$) and k_{el} (s^{-1}) is the rate constant of the first-
472 order gas elimination process. In the calculations, the rate constant determined for KCl in
473 MPC90 is used for the elimination process. The results of the modelling are shown in Figure
474 7. The red profile in Figure 7(A) represents 0.2% MPC90 and the black profile is the
475 simulation with a k_{gen} (gas generation rate) of $1.54 \times 10^{-3} s^{-1}$, derived from MPC90 terminal
476 gas disappearance rate and k_{el} (gas elimination rate) is $2.82 \times 10^{-3} s^{-1}$, derived from KCl gas
477 elimination rate in MPC90 solution. Figure 7 (B) shows the log plot of the data in Figure 7
478 (A). The AUV was calculated to be 7.87 mL.s. The total amount of gas generated was $2.22 \times$
479 10^{-2} mL, calculated as $AUV \times k_{el}$; note that $k_{el} > k_{gen}$, implying flip-flop characteristics of gas
480 production and elimination. The total amount of occluded gas in the MPC90 sample used in
481 the experiment was 2.31×10^{-2} mL. This was estimated for a 0.2% MPC90 system in 25 mL
482 of water from the occluded air value in Table 1. The total amount of gas generated during
483 powder rehydration (estimated from BARDS data) is in very close agreement with the
484 occluded air content of the powder.

485

486 **[Figure 7 about here]**

487

488 A similar set of experiments with rehydration of MPC90 in water and KCl in rehydrated
489 MPC90, but performed under slightly altered conditions (i.e., using a glass vessel with
490 different dimensions and therefore slightly different solution mixing dynamics) yielded
491 different gas elimination rate constants. However, the total amount of compressible gas
492 calculated was the same as found with the other experiment and again corresponded to the
493 value for occluded gas in MPC90. The results of the experiment are shown in Figure 7 (C)
494 and (D). The red profile in Figure 7 (C) represents 0.2% MPC90 and the black profile the
495 simulation with k_{gen} of $1.99 \times 10^{-3} \text{ s}^{-1}$, derived from MPC90 terminal gas disappearance rate.
496 The value for k_{el} of $5.17 \times 10^{-3} \text{ s}^{-1}$ was derived from the KCl gas elimination rate in MPC90
497 solution. The AUV was calculated to be 4.36 mL.s and the total amount of gas generated was
498 $2.25 \times 10^{-2} \text{ mL}$, calculated as $AUV \times k_{el}$; flip-flop characteristics are implied again as $k_{el} >$
499 k_{gen} . The calculated total gas volume was in excellent agreement with the total amount of
500 occluded gas in the MPC90 sample used in the experiment ($2.31 \times 10^{-2} \text{ mL}$). The
501 calculations demonstrate that the two experiments are in good agreement in terms of the total
502 amount of gas generated. The value is therefore independent of the differences in response
503 observed between the two experiments and the different rate constants used in the
504 calculations.

505 Crucially, the values calculated using flip-flop kinetics for the amount of gas
506 generated during the rehydration of MPC90 indicate that the gas detected by BARDS
507 originates exclusively from the occluded gas fraction of the powder. Thus, when considering
508 the BARDS spectra for the MPC90, the influence of interstitial air can be considered
509 negligible, which allows isolation of the gas generation phenomenon as one of water transfer
510 into powder particles.

511

512

513 3. 8 Microstructure of dry MPC powders

514 SEM micrographs of representative low- (MPC35), intermediate- (MPC70) and high-
515 protein (MPC90) powders are shown in Figure 8. In agreement with particle size data (Table
516 1), there was a greater quantity of small particles in the MPC90, while the MPC35 and MPC70

517 were similar in this respect. Increasing protein content was associated with two distinct
518 morphological changes, the smoothening of particle surfaces and partial deflation of the surface
519 towards the particle interior. Smooth particle surfaces may be attributable to differences in
520 compositional homogeneity of the particle surfaces in the MPC powders. Kelly *et al.* (2015)
521 determined that protein constituted 63, 79 and 93% of the surface of MPC35, MPC70 and
522 MPC90 (same sample set), respectively; the surface of MPC35 contained a large quantity of
523 lactose (31%), while the surface of MPC90 contained <1% lactose. The deflation effect is
524 characteristic of casein-dominant dairy powders, such as MPCs and MCCs, and is not observed
525 for whey protein-dominant powders (Sadek *et al.*, 2016). It is generally associated with
526 powders containing high levels of occluded air, which is the case for MPC70 and, in particular,
527 MPC90 (Table 1), where distinct internal air vacuoles and external protein layers are present.
528 Recent studies suggest that highly concentrated casein suspensions undergo a form of gelation
529 during drying, and that this surface gel has distinct mechanical properties which result in this
530 final deflated or buckled powder particle shape (Sadek *et al.*, 2016).

531

532 **[Figure 7 about here]**

533

534

535 *3.9 Microstructure of MPC powders during rehydration*

536 To investigate the dispersion state of powder particles during rehydration, cryo-SEM
537 was used to visualise powders with fast (MPC35) and slow (MPC90) water transfer
538 characteristics. Cryo-SEM micrographs of MPC35 and MPC90 powders during rehydration are
539 shown in Figure 9. The three different time points represent pre-steady-state for both powders
540 (100 s), steady state for MPC35 and not MPC90 (1000 s), and post-steady-state for both
541 powders (3000 s), as determined by BARDS.

542

543 **[Figure 9 about here]**

544

545 After a short period of 100 s, partially-dispersed or fragmented particles were present in
546 both MPC35 and MPC90; however, the latter also contained intact powder particles, similar in

547 size (~20 μm) to the particles observed in the corresponding micrographs for dry powders
548 (Figure 8). When the powders were rehydrated for 1000 s, numerous small, distinct particles
549 predominated in MPC35 which were ~1 μm in size, while several larger particles (~5 μm)
550 remained in MPC90 with few distinct particles in general being visible. After 3000 s of
551 rehydration, the majority of particles in MPC35 were <1 μm , with a minor distribution of
552 micron-sized particles, while, in contrast, MPC90 was still populated largely by particles >1
553 μm ; in addition, ring-link structures can be seen in Figure 9 (1C), consistent with the possible
554 presence of hydrated but undispersed powder particles, as suggested previously (Mimouni *et*
555 *al.*, 2009; Crowley *et al.*, 2015). Figure 9 also shows the BARDS frequency-time profiles for
556 all seven MPCs with a sample mass of 50 mg (0.2%). The BARDS measurement times
557 corresponding to the rehydration times where the micrographs were captured are indicated. It
558 can be seen in Figure 9 that for an equivalent stage of water transfer, such as the steady-state of
559 all MPCs at 3000 s, different dispersion states can exist. This is because BARDS is a technique
560 that detects the completion of water transfer into particles but not necessarily the disappearance
561 of granular particle structures. However, both water transfer and dispersion occur
562 simultaneously, indicating that a possible relationship between the two phenomena exists; this
563 is expanded in Section 4.

564

565

566 4. Discussion

567 This is the first study reporting on the gas release/water transfer properties of a full range of
568 MPCs, ranging from low to high protein. Results from BARDS analysis indicated that water
569 transfer into MPC powder particles became inhibited as the protein content of the MPC
570 powders increased (Figures 2, 3, Table 1). For example, rehydration of MPC35 yielded a
571 minimal BARDS response and a rapid return to steady-state (<400 s), while the time (60 min)
572 required to reach steady-state for MPC90 (0.2%) (Figure 2) is unprecedented in its length
573 compared to previous BARDS studies (Fitzpatrick *et al.*, 2012a, 2012b, 2013, 2014). Release
574 of gas from powders can be used to indirectly investigate water transfer in dairy powders
575 (Richard *et al.*, 2012; Hauser and Amamcharla, 2016;). Gas in powders consists of interstitial
576 (between particles) and occluded (within particles) air. In the samples studied, greater
577 quantities of both were present in high-protein powders such as MPC90 (Table 1), but flip-
578 flop kinetic analysis of MPC90 (0.2%) indicated that only occluded air was detected by

579 BARDS (see Section 3.7); thus, the higher levels of occluded air in high-protein MPCs were
580 responsible for the greater total volume of gas which was released during their rehydration
581 (Figure 3). The volume of compressible gas generated was, as would be expected, in
582 proportion to the mass of powder added to the water (Figure 5).

583 Figure 3B demonstrates that at concentrations of 0.2% gas was released much more
584 slowly into the solvent during the rehydration of high-protein MPCs (MPC80, MPC85 and
585 MPC90) compared to the lower protein MPCs. Gas generation in these high-protein MPCs
586 was partially delayed by slow wetting, due to their high air content and consequent poor
587 sinkability (Table 1) but also the high hydrophobicity indicated by the large contact angle
588 formed between the powders and water (Crowley *et al.*, 2015). However, although wetting
589 lasted 200 s for these powders, gas generation was still dominating over gas elimination until
590 500 s in the BARDS spectra of 0.2% MPC80, MPC85 and MPC90, confirming that water
591 transfer into particles continued after wetting. For MPC80 and MPC85, Figure 3C and Table
592 2 show that the gas disappearance phase was intermediate among the powders and similar to
593 MPCs which did not exhibit inhibited water transfer (MPC60, MPC70). For this reason,
594 inhibited water transfer was not considered to strongly affect the gas disappearance behaviour
595 in MPC60, MPC70, MPC80, MPC85; instead, the impeding influence of solubilised protein
596 on bubble escape (Ybert and di Meaglio, 1998) was considered to define gas elimination in
597 these systems. The more rapid gas disappearance for MPC35 and MPC50, which displayed
598 similarly fast water transfer to MPC60 and MPC70, was likely due to the lower levels of
599 protein available to impede bubble escape. Indeed, it was demonstrated in this study that the
600 properties of a protein solution can influence bubble escape (Fig. 6) during the rehydration of
601 a solute, which is an important consideration when conducting rehydration assessments using
602 BARDS and other sound-based methods.

603 The rate of gas disappearance for MPC90 was the slowest of all the powders, which
604 suggested that water transfer into powder particles was still influential during the gas
605 disappearance phase; however, the influence of water transfer and solvent properties needed
606 to be differentiated. Although certain physicochemical properties of a 0.2% MPC90
607 suspension (which had undergone complete water transfer) affected gas bubble elimination
608 (Figure 6), slow water transfer into the powder particles was determined to be the major
609 factor limiting gas disappearance from the solvent. The influence of the markedly slow
610 process of water transfer into particles therefore persisted throughout the, intermediate and

611 late stages in the BARDS spectra for MPC90. A study of concentration-dependency,
612 however, revealed differences between MPC80 and MPC90, which has also been observed
613 previously for their dispersion characteristics (Crowley *et al.*, 2015). For MPC80 (gas
614 elimination-limiting), the rate of gas disappearance was affected by concentration effects
615 such as soluble protein, but this was not the case for MPC90 (gas generation-limiting), as the
616 process of water transfer was not influenced by concentration (Figure 6).

617 Cryo-SEM micrographs indicated that the dispersion of particles on the transition
618 from a dry powder (Figure 8) into a rehydrated solution (Figure 9) was slower and less
619 complete for MPC90 compared to MPC35, due to the poor dispersibility of particles in high-
620 protein MPCs (Fang *et al.*, 2011; Mimouni *et al.*, 2009; Crowley *et al.*, 2015; Li *et al.*, 2016).
621 The cryo-SEM micrographs can be compared to the BARDS spectra in Figure 10. At 100 s of
622 rehydration, MPC35 had a limited BARDS frequency deflection due to the quick dispersion of
623 particles capable of releasing the minor levels of occluded air present (Table 1). As large
624 structures capable of entrapping air were no longer present due to effective dispersion, MPC35
625 rapidly reached steady state before 1000 s had elapsed; conversely, water transfer into MPC90
626 particles was slow, and the particles themselves underwent more limited dispersion, resulting in
627 continued air release from the particles. When particles in both MPCs had undergone
628 significant dispersion into smaller fragments and dissolution into component molecules, neither
629 powder exhibited any air release (~3000 s). However, at this point, the rehydration state of both
630 powders cannot be considered equivalent, as it is clear that much larger particle structures
631 remained in the MPC90. The MPC35 primarily consisted of particles <1 μm , which would be
632 expected for the nanoscale proteins present in milk. On the other hand, MPC90 contained a
633 substantial proportion of micron-sized particles, which were presumably undispersed powder
634 particle fragments.

635 BARDS data indicating inhibited water transfer during the rehydration of high-protein
636 MPCs, particularly MPC90, must be considered in the context of a growing body of evidence
637 supporting the presence of a 'skin' of inter-linked casein micelles at the surface of high-
638 protein MPC particles, which has been linked with the poor rehydration characteristics of
639 these powders (McKenna, 2000; Mimouni *et al.*, 2010b; Fyfe *et al.*, 2011; Crowley *et al.*,
640 2016; Ji *et al.*, 2016). The results of the present study suggest that this skin of inter-packed
641 casein micelles may act as a barrier which reduces the rate of water transfer into particles
642 during the rehydration of high-protein MPCs. The protein:lactose ratio at the surface of the
643 MPC particles studied here decreased significantly as the protein content of the powders

644 increased (Kelly *et al.*, 2015). This altered surface composition may have removed lactose as
645 a hydrophilic channel for effective water transfer into the particle resulting in a relatively
646 homogenous and hydrophobic particle surface (Fyfe *et al.*, 2011; Crowley *et al.*, 2015). The
647 absence of lactose as a physical ‘spacer’ may also have promoted the tendency for proteins-
648 protein interactions resulting in cohesive protein skin (Anema, 2006; Havea, 2006). The
649 BARDS data and cryo-SEM micrographs in Figures 8, 9 and 10 strongly support that both
650 water transfer and dispersibility are impaired in high-protein MPCs. This has also been found
651 for MCCs, where a link between rapid water transfer and effective dispersion has been
652 proposed (Richard *et al.*, 2012) and demonstrated (Bouvier *et al.*, 2013).

653 The nature of the relationship between water transfer and dispersion has yet to be
654 established, although it is evident from the present study that MPCs with poor water transfer
655 properties also have poor dispersion characteristics. One possibility is that incomplete water
656 transfer results in regions of the particles remaining effectively ‘dry’, thereby limiting their
657 ability to attain the molecular mobility necessary to disperse effectively. This concept is
658 illustrated in Figure 10 with corresponding BARDS frequency profile for MPC90, which
659 shows how the presence of dry regions near the internal air vacuole of the particle could
660 result in the predominance of uni-directional (towards the bulk solvent) dispersion, where
661 components in immediate contact with the solvent are released first by an erosion-like
662 process. Transfer of water through the protein skin during rehydration could eventually
663 expose dry regions to a second solvent-front located in the interior of the particle. The
664 presence of these two solvent-fronts would then promote collapse of the particle through
665 multi-directional (towards the particle interior and the bulk solvent) dispersion.

666

667 **5. Conclusion**

668 BARDS was demonstrated to be an effective method for discriminating between MPC
669 powders with different rehydration characteristics. The BARDS experiments only required 25
670 mL of water and 10-50 mg of each MPC (0.04-0.20%), which minimises greatly the
671 quantities of powder required for comparable tests. An additional advantage is that BARDS is
672 non-invasive, as acoustic responses are derived from a non-contact microphone rather than a
673 submerged probe. MPC35, MPC50, MPC60 and MPC70 exhibited similar water transfer
674 properties, and differences in their BARDS spectra were primarily caused by their different
675 air contents and the effect of increasing protein content on bubble escape. High-protein MPC
676 powders (MPC80, MPC85 and MPC90) exhibited a characteristic BARDS response during

677 rehydration involving a prolonged period of gas generation to reach a maximum solvent
678 compressibility, due mainly to inhibited water transfer into the powder particles. The period
679 of gas generation during the rehydration of high-protein MPCs was followed by a prolonged
680 return to steady-state equilibrium; the disappearance of gas from the solvent during this phase
681 was influenced by the impeding effect of soluble protein on bubble escape; however, for
682 MPC90, inhibited water transfer was still dominant during gas disappearance. The water
683 transfer properties of high-protein MPCs were poor, but they were exceptionally poor for
684 MPC90. BARDS is one of the few techniques currently available which facilitates the
685 dynamic monitoring of water transfer during powder rehydration. Further BARDS studies
686 will focus on the effect of varying solvent composition and temperature of rehydration on
687 water transfer properties. BARDS may also be an attractive option for identifying defects in
688 the rehydration characteristics of high-protein dairy powders caused by process- or storage-
689 induced degradative changes.

690

691

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701

702

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1 **Figure Legends**

2 **Figure 1.** Principal of BARDS analysis as applied to MPC rehydration: Panel 1, Schematic of
3 the BARDS Instrument; Panel 2, Addition of MPC powder to the BARDS dissolution vessel;
4 Panel 3, Water/Air transfer of an MPC particle during rehydration and raw BARDS spectra
5 of the rehydration of 50 mg of MPC90 in 25 mL of deionised water at 22 °C.

6 **Figure 2.** Comparison of BARDS spectra of all MPC powders dissolved in 25 mL deionised
7 water at 22 °C with a consistent sample concentration of 0.2% (w/v).

8 **Figure 3.** Concentration-dependence of BARDS frequency response during rehydration of
9 MPC in deionised water at 22 °C: (A) MPC35; (B) MPC70; (C) MPC80; (D) MPC90.

10 **Figure 4.** Gas volume-time plots derived from BARDS frequency data: (A) Gas volume plots
11 of all MPCs tested during rehydration of 50 mg MPC in 25 mL water at 22 °C; (B)
12 Magnification of gas volume profile during the initial phase shown in (A); (C) A log plot of
13 the gas volumes in (A), the slopes of which are used to calculate the BARDS first-order rate
14 constants (k).

15 **Figure 5.** Gas volume plots for (A) MPC35, (B) MPC 70, (C) MPC80 and (D) MPC90, using
16 a logarithmic scale for the gas volume.

17 **Figure 6.** Investigation of influence of solvent properties on gas disappearance: (A)
18 Frequency-time plot of MPC dissolved in water, KCl dissolved in water or KCl dissolved in
19 MPC90 solution; (B) log plot of gas volume data derived from (A).

20 **Figure 7.** Modelling of BARDS data using flip-flop kinetics: (A) Modelling of 0.2% MPC90
21 data, as presented in Fig. 3A. (B) Log plot and simulation of the data in (A). (C) Modelling of
22 0.2% MPC90 data obtained under slightly different conditions and (D) log plot and
23 simulation of the data in (C).

24 **Figure 8.** Cryo-SEM micrographs of dry (1) MPC35, (2) MPC70 and (3) MPC90 at
25 magnifications of (A) 500× and (B) 2500×, with scale bars of 50 and 10 μm, respectively, for
26 the magnifications.

27 **Figure 9.** BARDS spectra of all MPCs added at 0.2% w/v and cryo-SEM micrographs of (1)
28 MPC35 and (2) MPC90 after rehydration for (A) 100 s, (B) 1000 s, and (C) 3000 s.

29 Corresponding rehydration times in the BARDS spectra and micrographs are indicated for
30 comparison.

31 **Figure 10.** Schematic representation of protein 'skin' at the surface of a primary powder
32 particle in a high-protein MPCs and the hypothesised relationship between inhibited water
33 transfer and the poor dispersion of these particles. A BARDS profile for MPC90 is shown.

Table 1. Composition and physical properties of milk protein concentrates (MPCs). Data presented are the means of duplicate analysis, with the exception of lactose, which was the result of a single analysis.^a

Table 2. Results from kinetic analysis of log gas volume-time plots taken from BARDS measurement of different MPC powders.

Tables

Table 1. Composition and physical properties of milk protein concentrates (MPCs). Data presented are the means of duplicate analysis, with the exception of lactose, which was the result of a single analysis.^a

	Composition				Physical properties		
	Protein	Lactose	Ash	Fat	d ₅₀ ^b	Interstitial air	Occluded air
	(% , w/w)				(μm)	(ml 100 g ⁻¹)	
MPC35	35.4	49.6	8.06	0.5	35.3	98	18.1
MPC50	49.9	35.8	7.75	0.8	43.0	88	14.1
MPC60	60.8	24.5	7.74	1.5	48.9	95	21.4
MPC70	68.3	18.0	7.99	1.2	39.6	111	23.0
MPC80	79.1	6.36	7.69	1.7	27.9	206	53.7
MPC85	84.0	1.81	7.54	1.2	26.1	229	47.2
MPC90	85.9	0.37	7.59	1.6	26.8	230	46.2

^a taken from Crowley *et al.* (2014a).

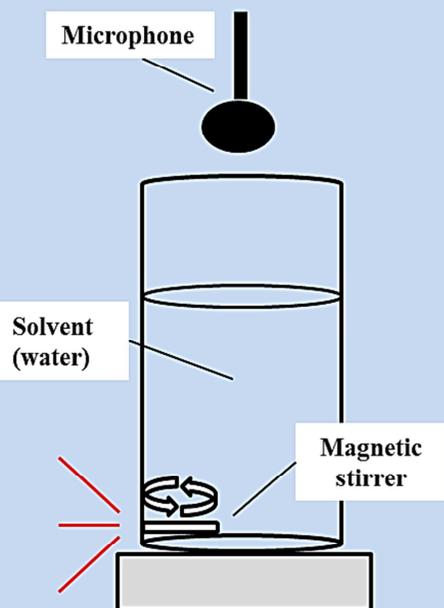
^b Particle size below which 50% of material volume exists – median.

Table 2. Results from kinetic analysis of log gas volume-time plots taken from BARDS measurement of different MPC powders.

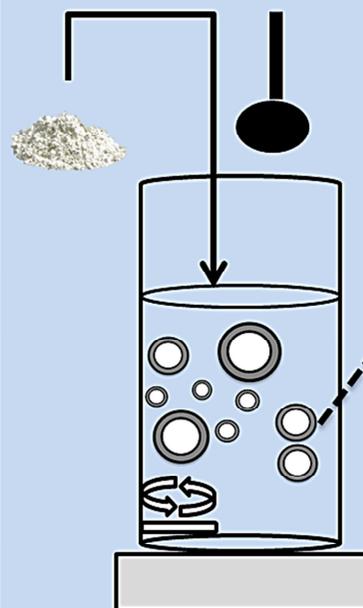
Compressible gas volume disappearance rate constant: k (s^{-1})														
Conc.	MPC35	fit	MPC50	fit	MPC60	fit	MPC70	fit	MPC80	fit	MPC85	fit	MPC90	fit
% (w/v)	k (s^{-1})	range (s)	k (s^{-1})	range (s)	k (s^{-1})	range (s)	k (s^{-1})	range (s)						
0.2	1.0E-02	220 -500	2.5E-02	40 -160	3.7E-03	240 -1500	6.6E-03	70 - 300	3.4E-03	500 -2000	3.88E-03	800 - 1800	1.9E-03	500 -2500
			9.5E-03	160 - 300			3.7E-03	400 - 1400						
0.16	1.0E-02	220 -500					1.3E-02	120 - 200					1.8E-03	400 -2700
							7.9E-03	220 - 500						
0.12	1.0E-02	220 -500					1.3E-02	120 - 200	4.3E-03	400 -1300			1.8E-03	400 -2100
							7.9E-03	220 - 500						
0.08	4.4E-02	60 -90					1.6E-02	120 - 220	4.9E-03	400 -1200			2.1E-03	700 -2400
	1.0E-02	120 -260					7.0E-03	240 - 500						
0.04	5.3E-02	40 -90					1.1E-02	60 - 180	6.7E-03	300 -600			2.8E-03	600 -1500
							7.1E-03	260 - 400	9.8E-03	100 -220				

Use of BARDS to monitor water transfer phenomena in MPC powders over time

1. Contact between a magnetic stirring bar and the vessel wall induces acoustic resonances which are detected by a microphone located above the vessel.



2. Addition of powder to the solvent introduces particles which release air as they are penetrated by water. Alterations to the acoustic resonance profile are measured over time.



3. Release of air changes the compressibility of the solvent, which results in a frequency profile that undergoes time-dependant changes, and is indicative of water transfer.

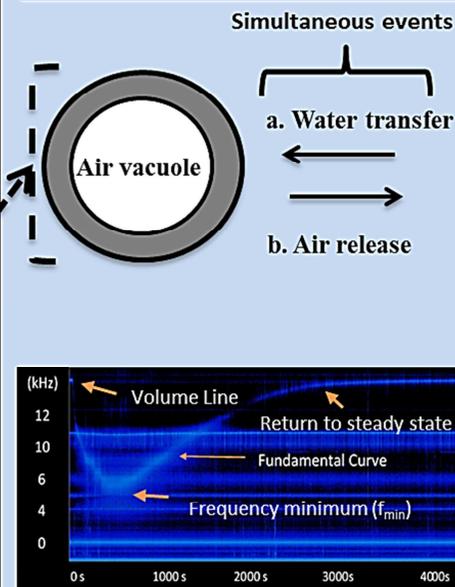


Figure 1.

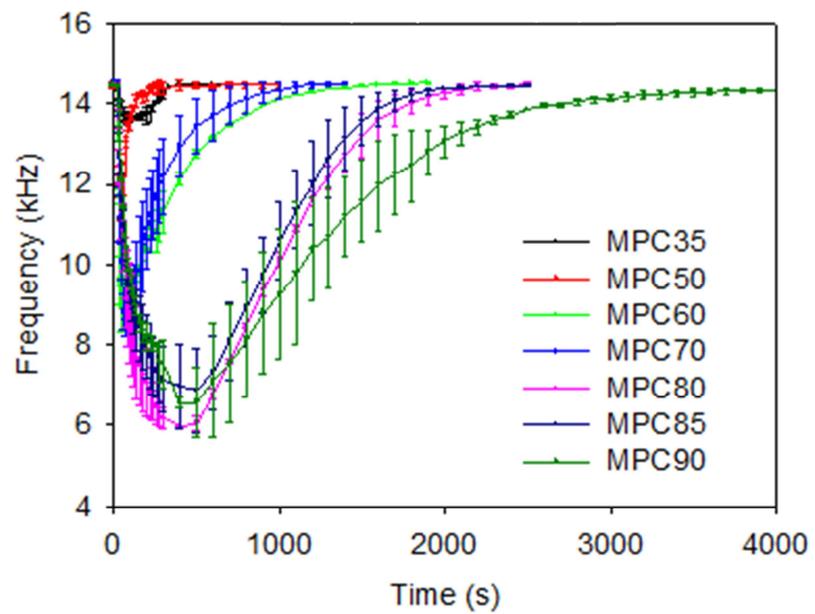


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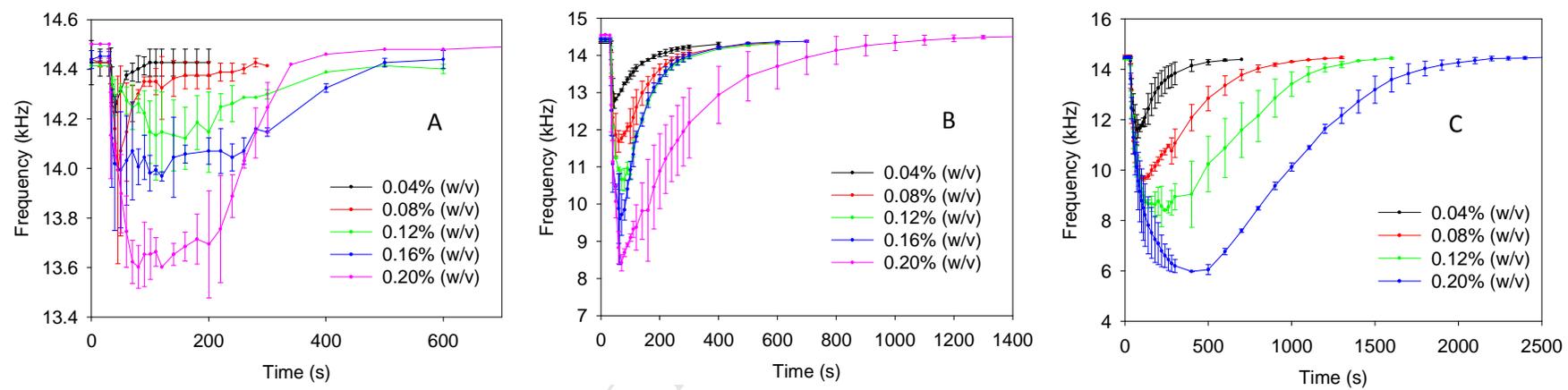


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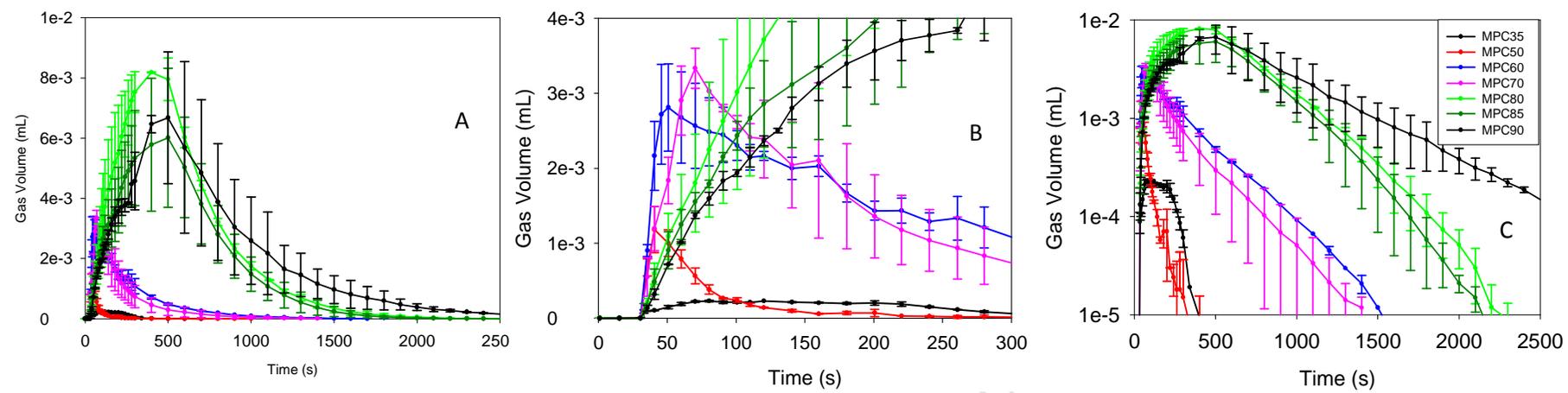


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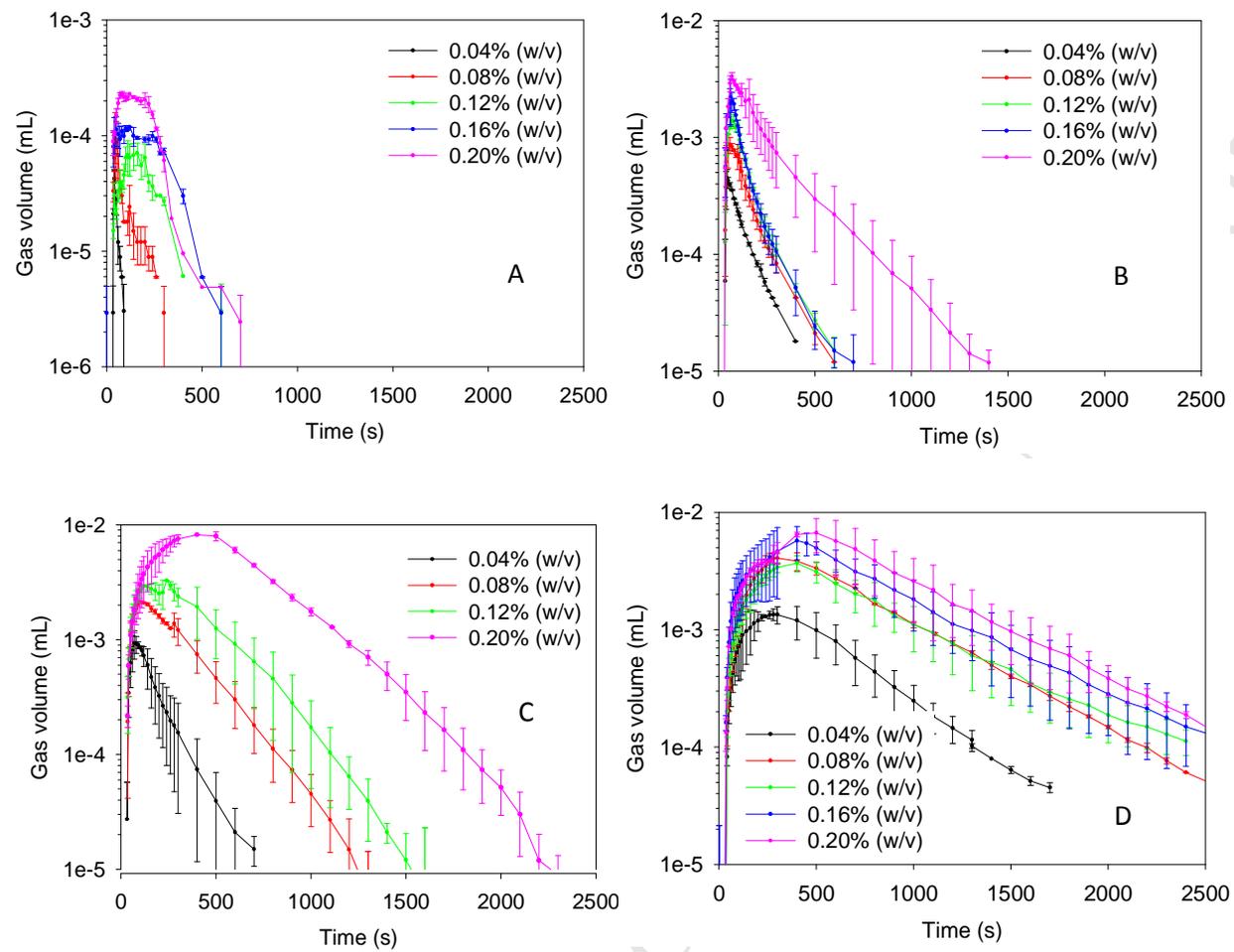


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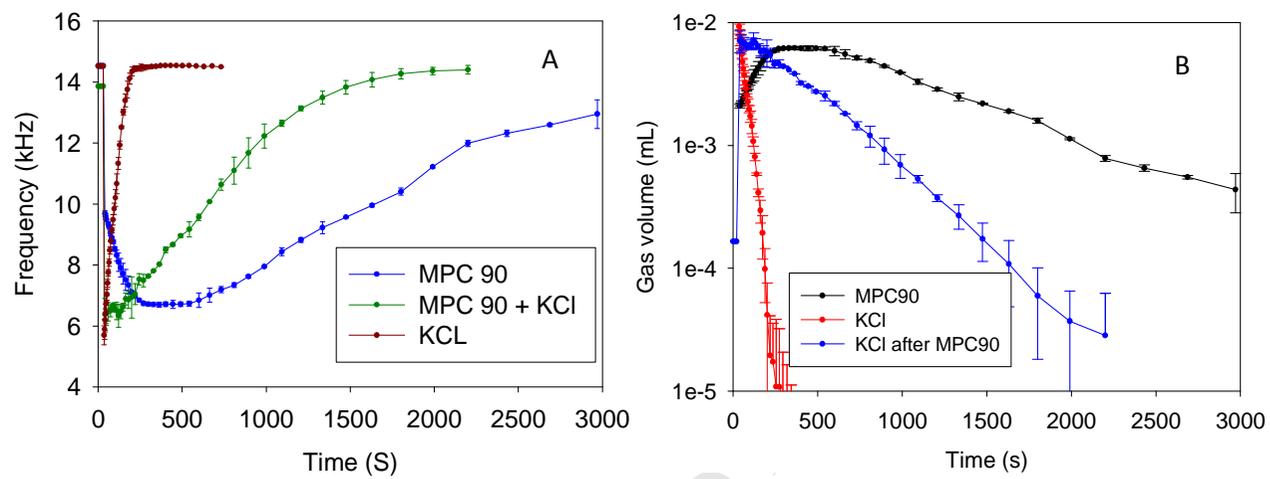
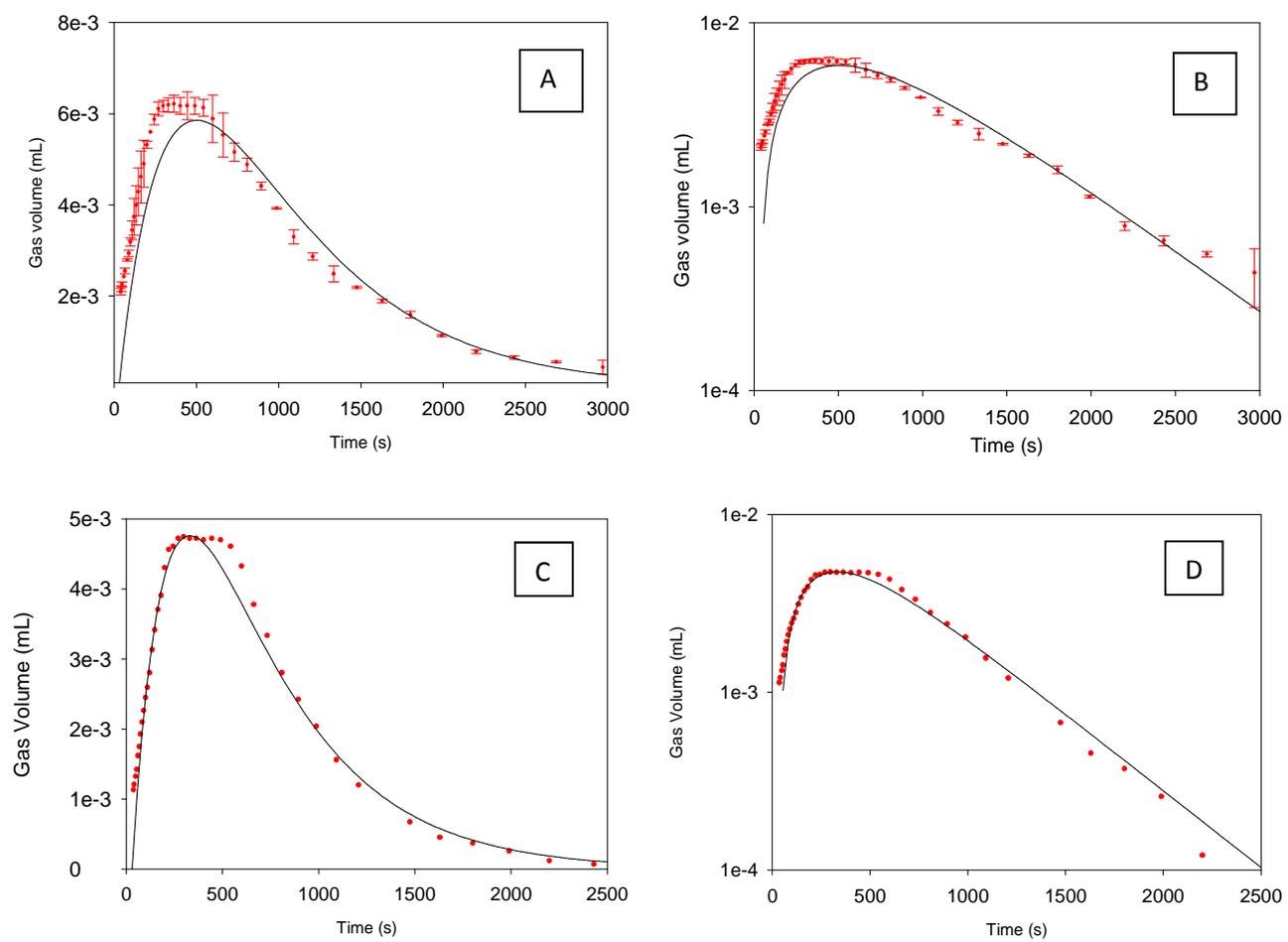


Figure 6.

**Figure 7.**

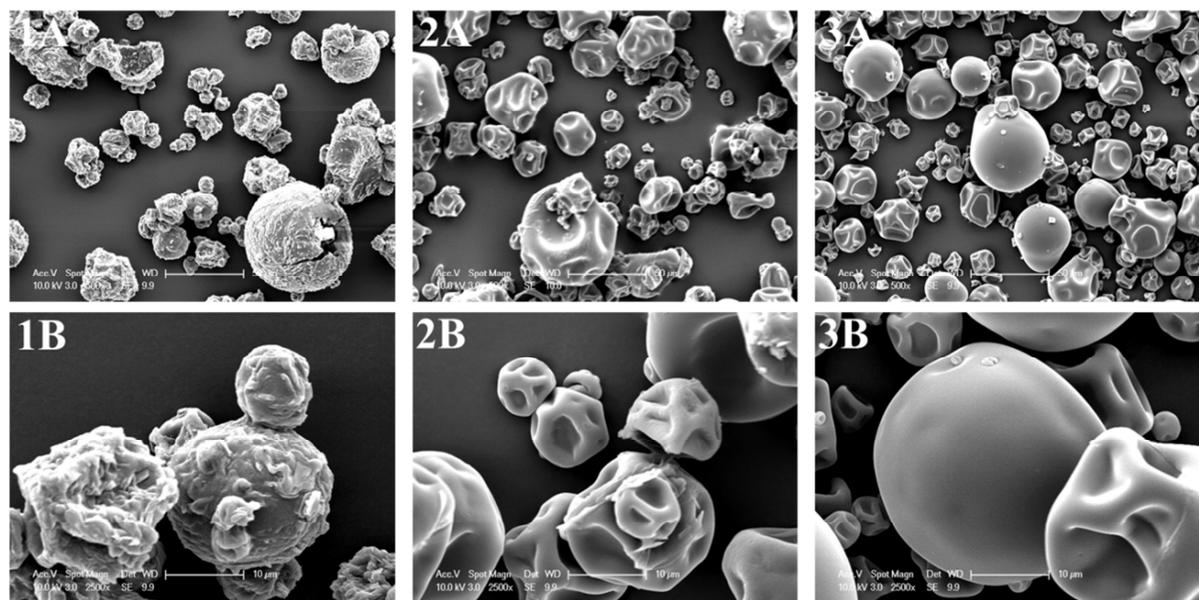


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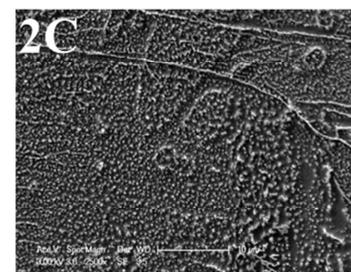
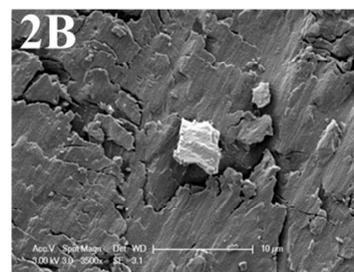
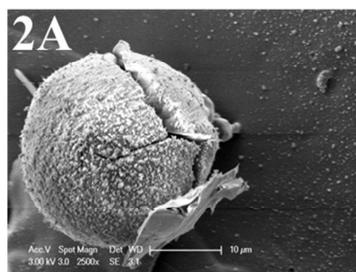
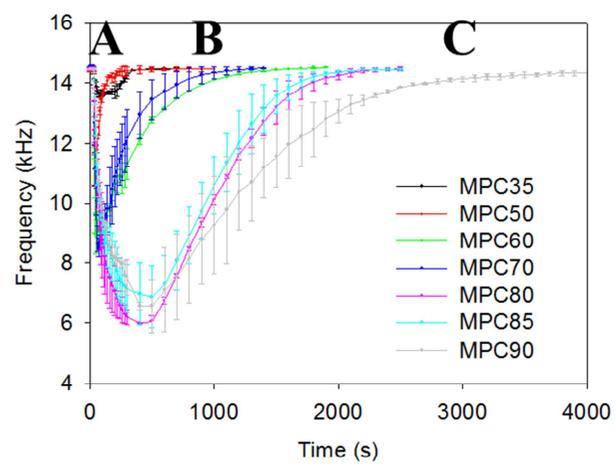
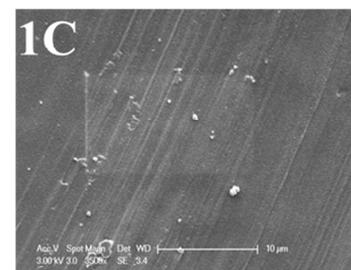
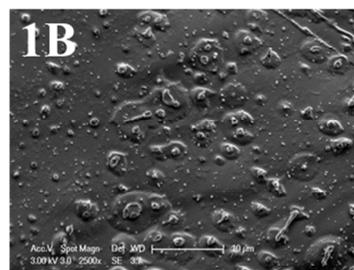
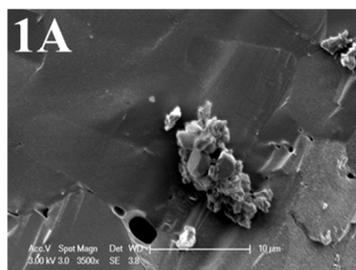


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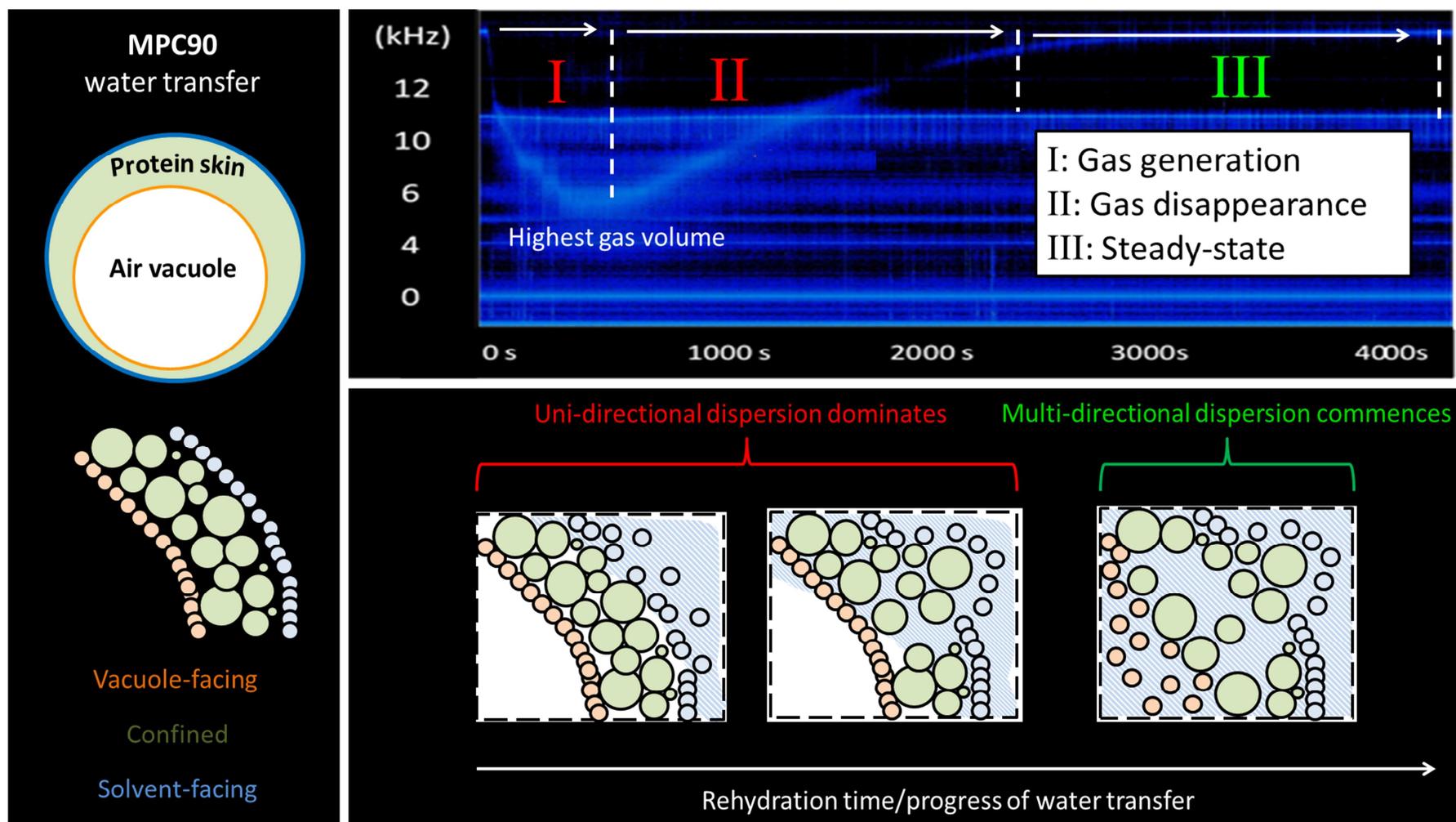


Figure 10.

Highlights

- BARDS measured changes in gas volume during MPC rehydration
- Gas generation/escape rates decreased with increasing protein content
- Occluded air from particles constituted the gas generated
- BARDS indicates water transfer was markedly inhibited in MPC90
- The hydration process of high MPC samples have been quantitatively modelled.
- Cryo-SEM confirmed slow water transfer/poor dispersion link