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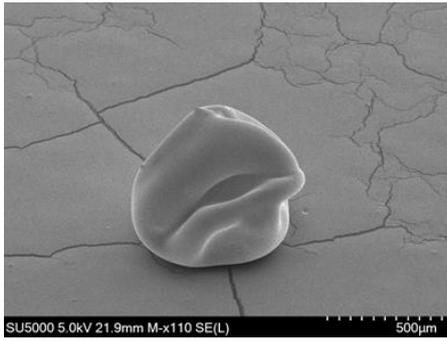
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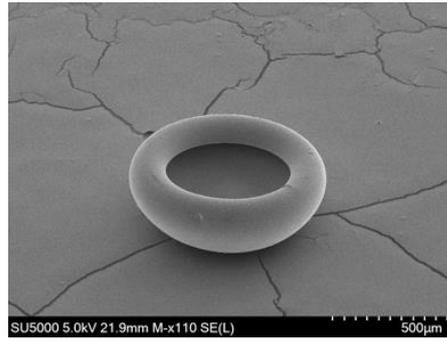
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Single droplet drying of whey protein dispersions



Soluble whey protein aggregate particle
Initial colloidal size = 50 nm



Fractal whey protein microgel particle
Initial colloidal size = 300 nm

1 Morphology development in single drop drying for native and aggregated whey protein dispersions

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9 Abstract

10 Native and aggregated whey proteins (WP) are used in food and pharmaceutical applications as
11 stabilizers, thickeners and carriers. For increasing shelf-life and facilitating transportation, WP are
12 transformed into powders by spray-drying. Powder functional properties strongly depend on the final
13 particle morphology. Focusing on colloidal aspects of drying, the goal of this work is to: (i) investigate
14 morphology development during single drop drying of native and aggregated whey protein
15 dispersions; (ii) use structure-mechanical parameters to predict the final morphology. Results showed
16 evaporation rate and morphology development characteristic times are not affected significantly by
17 colloidal size. However, the final morphology of particles depends on WP colloidal size. For small
18 colloids, particles are shriveled, while their shape is cup-like for larger colloids. Structure–mechanical
19 parameters allowed predicting a buckled/shriveled morphology in agreement with experimental
20 observations. Specifically, predictions anticipated the formation of a solid shell at the particle surface,
21 which is compressed during drying, as a result of colloidal interactions being dominated by van der
22 Waals forces. This work provides a rationalization of morphology development of WP particles. **In**
23 **addition, the work suggests that the elastic – or gel – formation, that is governed by the permeation,**
24 **may be very different depending on the permeability of the proteins gel. The collapse from a dispersion**
25 **to an elastic gel may be responsible for the shriveled to buckled transition.** The work shows that diverse
26 final morphologies can be achieved using same drying conditions and composition, while **only** changing
27 the degree of colloidal aggregation.

28 1.1 Introduction

29 The control of particle morphology during the spray drying of food powders is one of the key factors
30 affecting powder properties in food applications, under dry and reconstituted states. The dry powder
31 properties include powder density, porosity, pore size distribution and flowability. The **reconstituted**
32 **powder properties are decided based on the target final product properties. They depend on** particle
33 size distribution, the particle structure, as well as the wetting and re-dispersion properties, which can
34 follow complex mechanisms. This is true in particular for **dairy based** powders [1], [2], [3], **which are**
35 **the most common powder in the food industry. Typical dairy based powders are skim and whole milk,**
36 **high fat and whey protein powders. They are used for preparation of instant drinks, or as ingredients**
37 **i.e. in cheese, chocolate and baking products [4]. Reconstitution of dairy based powders is a slow and**
38 **complex mechanism** [5], [6]. **Recent works showed that full powder reconstitution may be slower than**
39 **usually described, and indeed depends on the drying conditions – whereby particulate structures can**
40 **take up to over an hour to fully re-disperse and dissolve. Crowley et al. [7] showed that the lactose to**
41 **protein ratio strongly influences particle morphology, while low lactose concentration was shown to**
42 **imply buckled particle or vacuole type morphologies, along with slower dissolution. Final particle**
43 **morphology depends also on protein composition. Sadek et al. [8] and Lanotte et al. [9] demonstrated**
44 **that whey protein particles are spherical and hollow, native casein particles are shriveled, and protein**

45 mixtures leads to hybrid particle morphologies. In addition to this, Fang et al. [10] showed the drying
 46 rate as a critical factor affecting particle morphology. The goal of the present work is to attempt
 47 rationalizing the behavior of well-defined dispersions of protein aggregates in their single drop drying
 48 behavior: drying rate, kinetics and type of morphologies developing. The reference system is a solution
 49 of non-aggregated whey protein, and the aggregate size and morphologies are varied. **Whey protein**
 50 **and their aggregate has been selected as reference system since they are of great interest for food**
 51 **application, especially for their emulsifying capacity [11], [12], [13].**

52 Generic research in the field of colloid suspension drying revealed the key mechanisms in play for
 53 systems whose behavior may be modeled with a few parameters describing colloidal interactions,
 54 Brownian motion, evaporation rate, as well as hydrodynamic stresses generated during drying. It was
 55 established from experiments using model colloidal suspensions, that the Péclet number, which
 56 balances the diffusion time of the colloidal particle (or molecule) over a characteristic distance with
 57 the drying time, is a key parameter for describing the drying mechanism. Under conditions of fast
 58 drying i.e. large Péclet number, the drop shrinkage is so fast that Brownian motion does not keep the
 59 colloids well dispersed within the drop, so they accumulate near the drop surface in the form of a
 60 concentrated suspension, forming a shell with solid mechanical properties. Depending on whether
 61 colloidal gelation occurred over nearly the whole drop volume before a solid shell could form or not,
 62 buckling will occur or not. Lintingre et al. [14] described in detail the dimensionless parameters, which
 63 allow for predicting the Péclet range for shell formation, as well as the conditions for buckling. It is
 64 known that aggregation can occur in the particle shell, even under conditions of colloidal interactions,
 65 which would be repulsive in the absence of a drying mechanism. The conditions of buckling are usually
 66 reached earlier for weaker repulsion or slight attraction between colloids, while strong attraction may
 67 lead to drop gelation and resistance to buckling.

68 1.2 Background

69 During drying, colloidal particles accumulate at the droplet-air interface, creating a solid shell that may
 70 buckle, leading to non-spherical morphologies. Such a phenomenon is explained by calculating
 71 relevant parameters as suggested by **Lintingre et al.** [14], such as the Péclet dimensionless number, Pe ,
 72 associated to the dispersed particles, the critical buckling pressure, P_{buck} , the capillary pressure, P_{cap} ,
 73 the Darcy's pressure, P_{Darcy} , and the Derjaguin Landau Verwey Overbeek (DLVO) potential, V_{DLVO} .

74 Pe is used to compare the effect of colloidal diffusion and drying rate (**detailed assumptions available**
 75 **in [14]**):

$$76 \quad Pe = \frac{R^2}{\tau_{dry}} \frac{6\pi\eta a}{kT} = m \frac{6\pi\eta a}{kT} \quad (\text{Eq. 1})$$

77 Where k is Boltzman's constant, T is the drying temperature, η is the solvent viscosity, a is the colloidal
 78 particle radius, τ_{dry} , is the drying time, R is the radius of the droplet (all in S.I. units). The constant m is
 79 introduced above and is equal to R^2 / τ_{dry} , namely evaporation rate.

80 If the Péclet number is larger than 1, the particles will accumulate and form a shell. Under the Darcy
 81 pressure, this shell may become an elastic solid. **This is often called sol-gel transition and may lead to**
 82 **fracture on macroscopic sample [15].** The particles forms an elastic – gel-like – shell, because the
 83 solvent continue to evaporate, the volume of liquid enclosed in the shell will decrease. Consequently,
 84 the shell will buckle, if the pressure in the shell exceed a critical value.

85 P_{buck} is the critical pressure above which the shell buckles and collapses:

$$86 \quad P_{buck} = 2E \left(\frac{h}{R}\right)^2 \sqrt{3(1 - \nu^2)} \quad (\text{Eq. 2})$$

87 E denotes the shell's Young modulus, ν is the Poisson ratio, h is its thickness. If the shell forms
 88 progressively, its thickness will increase with time. Since P_{buck} increases with the square of h , buckling
 89 will appear for very small h , as soon as the shell can be considered as an elastic body. It is a reasonable
 90 estimate to assume that the minimum value of h is $5a$, meaning 5 layers of particles are needed to
 91 consider that the shell is a solid body. In contrast, if the shell forms instantaneously, the thickness h
 92 may be large at the onset of the elastic body formation and equal to $R/5$ where R is the droplet radius
 93 for the shell thickness to occur. Above this thickness, no buckling will occur and a hollow sphere will
 94 be obtained.

95 The pressure exerted on the shell by the liquid inside is limited only by the water flow through the
 96 porous shell and thus by the capillary pressure, P_{cap} , which is maximum before penetration of air into
 97 the shell [16]:

$$98 \quad P_{\text{cap}} \approx \frac{4\gamma}{d} \quad (\text{Eq. 3})$$

99 Where γ is the droplet-air interfacial tension and d the pore diameter.

100 The formation of the shell itself is driven by the water gradient pressure across the shell, namely
 101 Darcy's pressure. P_{Darcy} brings together the colloidal particles promoting aggregation:

$$102 \quad P_{\text{Darcy}} = \frac{\eta v}{k_p} h \quad (\text{Eq. 4})$$

103 Where v is the relative velocity between liquid and particle shell, $v \approx R/\tau_{\text{dry}}$, k_p the shell permeability
 104 calculated as a function of the packing volume fraction and based on the Karman Cozeny expression,
 105 Φ_c , as:

$$106 \quad k_p = \frac{a^2(1-\Phi_c)^3}{45\Phi_c^2} \quad (\text{Eq. 5})$$

107 Lastly, P_{Darcy} succeeds to form an elastic shell only if it is able to overcome the electrostatic repulsion
 108 in between colloidal particles. At small distances, this repulsion is given by the Derjaguin Landau
 109 Verwey Overbeek (DLVO) potential, V_{DLVO} , superimposing charge induced repulsion and van der Waals
 110 attraction. At colloidal particle distances $r \ll a$ it is:

$$111 \quad V_{\text{DLVO}}(r) = 64\pi kT\Gamma^2 \rho_i \lambda_D^2 a e^{-r/\lambda_D} - \frac{Aa}{12r} \quad (\text{Eq. 6})$$

$$112 \quad \Gamma = \tanh\left(\frac{e\zeta}{4kT}\right) \quad (\text{Eq. 7})$$

113 Where e is the electron charge, ζ is the surface potential, A is the Hamaker van der Waals constant, ρ_i
 114 is the ion density in the solvent (mol m^{-3}). λ_D denotes the Debye length calculated as [17]:

$$115 \quad \lambda_D = \sqrt{\frac{\epsilon \mathfrak{R}T}{2 \rho F^2 I b^\ominus}} \quad (\text{Eq. 8})$$

116 Where ϵ is the dielectric constant, \mathfrak{R} is the ideal gas constant, ρ is the density of the solution, F is the
 117 Faraday's constant, and b^\ominus is the standard molality (mol kg^{-1}), I is the dimensionless ionic strength of
 118 the initial solution:

$$119 \quad I = \frac{1}{2} \sum_i z_i^2 \frac{b_i}{b^\ominus} \quad (\text{Eq. 9})$$

120 z_i denotes the charge number of an ion i , and b_i its molality.

121 Material and Methods

122 Materials

123 Whey protein isolate (WPI) powder (BiPRO, lot number LE 020-7-420) was purchased from AgroPur
124 Inc. (Jerome, USA). This product is obtained by ionic chromatography of ultrafiltered sweet whey in
125 order to remove caseino-glycomacropeptide and contains therefore no casein fractions [18].
126 According to the manufacturer, WPI powder has a composition of (in g/100g): 93.2 protein (Nx6.38),
127 4.4 moisture, 2.3 ash, and fat 0.5. Sodium chloride (NaCl), hydrochloride (HCl), sodium hydroxide
128 (NaOH) were purchased from VWR, Titrisol©, Sigma-Aldrich respectively.

129 Preparation of protein dispersions

130 WPI dispersions at 4 and 7 wt% solid content, which correspond to a protein content of 3.7 and 6.5
131 wt%, respectively. They were prepared using Milli-Q water (Millipore Synergy UV, Millipore AG,
132 Switzerland) in batches of 1L by stirring over night to ensure complete hydration. Three different types
133 of WPI aggregates were produced in a double-jacketed cylindrical vessel, equipped with a turbine
134 mixer (Janke & Kunkel RW 20, IKA Labortechnik, Germany), two water baths, one for heating and one
135 for cooling, and a temperature controller (TM-947SD, Lutron Electronic).

136 The WPI aggregates were produced according to the protocol of Schmitt et al. [18] and Phan-Xuan
137 [19]. The preparation of dispersions of WPI aggregates comprises:

- 138 (i) pH adjustment of the initial WPI dispersion by dropwise addition of 1 M HCl or NaOH under
139 vigorous stirring, the different conditions are described in Table 1;
- 140 (ii) WPI dispersions were transferred to the cylindrical vessel, and heated under stirring (200
141 rpm) to 85°C in about 15 min. The temperature was held for 10 min. Dispersions were then
142 rapidly cooled down using ice water until room temperature to stop the aggregation
143 process.

144 After aggregates were produced, dispersions were diluted to the same initial solid content of 4 wt%.
145 Dispersions with added salt were prepared right before experiments by adding NaCl in the
146 concentration of 1 wt%, 3 wt% and 6 wt% on a dry basis (db).

147 Colloidal size determination

148 The size measurements were performed using a Zeta sizer Nano ZS dynamic light scattering device
149 (Malvern Instruments, Worcestershire, U.K.), equipped with a He-Ne laser emitting a polarized light
150 beam of wavelength of 633nm. For the measurements, all protein dispersions were diluted 1:1000
151 using Milli-Q water. WPI dispersions were filtered prior measurement using a 0.1 and a 0.025 µm filter
152 (NC 03 Membrane Filters, Schleicher & Schuell, Germany) [20]. The measurements were performed in
153 10 mm disposable cuvettes at a temperature of 25°C. The scattered intensity fluctuations were
154 collected at a backscattering angle of 173°. The results were averaged over 3 runs and the time
155 correlation function of the scattered intensity was analyzed using the CONTIN method [21].

156 Single droplet drying

157 Drying experiments were conducted using a single droplet drying kinetics device, composed by a single
158 axial acoustic levitator (tec5 ultrasonic levitator, Germany), which works at a standard frequency of 58
159 kHz. It consists of a transducer that creates an acoustic wave, and a concave reflector that reflects it
160 back, in order to create a standing wave to allow droplet levitating (Figure 1). A free jet nozzle is
161 integrated into the reflector; it has a diameter of 1 mm and allows dry air to be blown upward around
162 the levitated droplet. The dry air was supplied by a compressed dry air cylinder. Measurements were
163 performed at constant airflow of 100 cm³min⁻¹ and at room temperature (23 ± 2°C). The distance
164 between reflector and transducer was 2 cm, and the power of the acoustic field was adjusted via HF
165 controller to ensure droplet stability. A 2 µl bubble-free droplet was inserted into the acoustic field
166 using a 100µl syringe (SGE, Australia). Morphological and size changes of the droplet during drying
167 were recorded using a Nikon camera D5300 (Tokyo, Japan) together with a macro lens Nikon Micro

168 Nikkor, 52 mm 1:1 (Tokyo, Japan). Two light sources were used depending on whether morphology or
169 size changes were investigated. A front light system was used to follow morphology during drying, it
170 includes two OSRAM Parathom® Advanced GU5.3 MR16 LED lights in position A. A back light system,
171 instead, was used to follow morphology changes, it consists of a green LED emitter (LZC-00MC40 RGB
172 LED, LedEng Inc.) controlled by a linear RGB LED Controller in position B. The generated images were
173 then analysed using image analysis as described in the next paragraph. All measurements were carried
174 out in triplicates.

175 **Evaporation rate calculation**

176 Pictures obtained using back lighting were used to measure the size change of each droplet during the
177 drying time using image analysis. Original images were cut into 500 x 500 pixels images and processed
178 using the software CellProfiler 2.2.0. The image analysis pipeline consists of the following sequence of
179 operations: Log transform (base 2), Invert, Identify Primary Objects, Measure Object Size Shape, Export
180 to Spread Sheet. The output comprises the object area in pixels, which was converted in real size using
181 the transducer as a reference size. Finally, the radius of the droplet was calculated and the evaporation
182 rate could be determined using the "radius squared law"[22]:

$$183 \quad r^2(t) = R_0^2 - mt \quad (\text{Eq. 10})$$

184 Where r is droplet radius as a function of the time, t ; R_0 is the initial droplet radius and m the
185 evaporation rate (all in S.I. units). The constant m is equal to R^2 / τ_{dry} as introduced above (see Eq. 1).
186 The evaporation rate was calculated only in the first 210 s of drying, **which is the period when all**
187 **measurements show a linear spherical shrinkage**. Initial volume of the droplets was also calculated
188 and only droplets in the range of $2 \pm 0.3 \mu\text{l}$ were used for the analysis.

189 **Morphology characterization**

190 Morphology changes over the drying time were investigated using pictures obtained by front lighting.
191 Morphology was characterized by two characteristic times, locking and buckling time, identified
192 visually as suggested by Both et al. [2]. The locking time is defined as the time when the droplet
193 becomes non-spherical, while the buckling time is when significant morphology development is
194 observed. Examples of locking and buckling time are reported in Figure 2.

195 **Scanning electron microscopy**

196 Selected dried particles were visualised with Scanning Electron Microscopy (SEM, Hitachi, SU5000).
197 Particles were placed on carbon tape and coated with a 10 nm layer of gold (CCU-010 Metal Safematic).
198 Images were obtained under vacuum at an accelerating voltage of 5 kV.

199 **Results**

200 **Colloidal dispersion characterization**

201 Three types of WPI aggregates, namely soluble **whey protein aggregates (WPA)**, **whey protein**
202 **microgels (WPM)** and fractal WPM, were produced according to the protocol reported previously.
203 **Using these conditions, more than 80% of the native whey proteins are converted into: microgels,**
204 **fractal aggregates of microgels or soluble fractal aggregates [18, 19]. The protein fraction coexisting**
205 **together with microgels is composed by soluble aggregates which are characterized by a fractal**
206 **dimension of about 1.8 [19].** Additionally, a WPI dispersion was prepared. Aggregated dispersions were
207 used with and without NaCl addition. Dispersions were characterized in terms of colloidal size and
208 results are reported in Table 2. Results show that the four dispersions have different average colloidal
209 sizes. WPI dispersions were characterized prior and after filtration.

210 The smallest colloids are WPA (49.2 nm), followed by WPI (181.2 nm), WPM (230 nm) and finally fractal
211 WPM (290.9 nm). Colloidal size of WPA, WPM and fractal WPM are in agreement with results reported
212 in literature. The expected range of WPA hydrodynamic diameter is from 43 to 58 nm [18], [23].
213 Instead, WPM and fractal WPM hydrodynamic diameters are expected in the range from 100 to 1000
214 nm [24], with fractal WPM being larger than WPM since they consist of WPM assembled into a self-
215 similar structure [25]. Addition of NaCl does not modify the colloidal size of aggregated protein
216 dispersion, since salt was added right before size and drying measurements to avoid exactly colloidal
217 size enlargement.

218 The hydrodynamic diameter of WPI results to be higher than expected due to the presence of self-
219 forming aggregates of WPI in water. The expected size was about 5-10 nm since it is composed by
220 native proteins [26], [27], as also confirmed by filtered WPI dispersions measurements. In this study,
221 we consider the average size of unfiltered WPI dispersions, since filtration was not performed prior
222 drying experiments.

223 **Single droplet drying**

224 Single droplet of protein dispersions were dried using the acoustic levitator droplet drying device.
225 Morphology development of a single droplet during the drying time was monitored to determine the
226 evaporation rate, as well as the locking and buckling times.

227 Evaporation rate of the constant drying rate period was evaluated and results are shown in Figure 3 as
228 a function of the colloidal diameter and added salt concentration. Ideal shrinkage occurs during the
229 constant drying period; hence, the “radius squared law” was applied. Results show that for all
230 dispersions, evaporation rate ranges from 4×10^{-10} to $8 \times 10^{-10} \text{ m}^2\text{s}^{-1}$. In case of 0 and 6 wt% (db) added
231 salt, the evaporation rate seems to increase with the colloidal size, however, no trend can be observed
232 in case of 1 and 3 wt% (db) added salt.

233 Figure 3 shows the locking and buckling time of single droplets as a function of colloidal size for no
234 added NaCl. For all solutions, average buckling time is always higher than average locking time, except
235 for soluble WPA, for which there is no difference between locking and buckling time. In addition to
236 this, buckling and locking time for WPA is above 12 min, instead in case of WPI, WPM and fractal WPM
237 they are lower than 8 min. In case of WPI, WPM and fractal WPM it seems that locking and buckling
238 time increase as a function of the colloidal size, suggesting that shape instability could be delayed by
239 enlarging the colloid particle. Addition of salt to WPI, WPM and fractal WPM solution does not seem
240 to affect the locking and buckling time, except for soluble WPA (Figure 4). For soluble WPA, addition
241 of salt seems to anticipate shape instability, especially in case of 1 wt% added NaCl.

242 The final morphology of single particles was observed using a macro camera and SEM (Figure 5).
243 Soluble WPA with no added salt exhibits a shriveled morphology, whereas WPI, WPM and fractal WPM
244 show a cup-like shape. Salt addition does not seem to affect final particle morphology of WPM and
245 fractal WPM. In case of soluble WPA, addition of NaCl leads to the formation of a shriveled particle
246 with a lower degree of invaginations. Hence, for small colloidal size particles are shriveled, while their
247 shape is cup-like for larger colloids. Morphology of WPI particles are in agreement with results
248 reported by Sadek et al [8] and Lanotte et al [9].

249 **Shell formation and mechanical properties**

250 In order to explain morphology development of single droplets during drying, parameters affecting
251 shell formation and mechanical properties are estimated. Pe is calculated considering $k = 1.38 \times 10^{-23} \text{ J}$
252 K^{-1} , $T = 298 \text{ K}$, because drying is performed at room temperature, and $\eta = 0.001 \text{ Pa}\cdot\text{s}$, since in this case
253 the solvent is water. The ratio R^2 / τ_{dry} is equal to the evaporation rate, m , calculated from Eq 10. P_{buck}

254 is estimated assuming $E = 10^4$ Pa [28], $\nu = 0.4$ [29], $h = 5a$, R is the droplet radius at the locking time. In
255 case of P_{cap} , $\gamma \approx 50$ mN.m⁻¹ [30] and pore diameter $d = a/5$. P_{Darcy} is estimated assuming a random close
256 packing volume fraction, $\Phi_c = 0.64$ [31], this assumption implies that no strong particulate gel forms
257 within the shell, or at least not before the shell reaches a particle concentration around random close
258 packing. V_{DLVO} is calculated considering $e = 1.6 \times 10^{-19}$ A s, $\zeta \approx -20$ mV [30], $A = 10^{-20}$ J, $\epsilon = 7.1 \times 10^{-10}$ J⁻¹C²m⁻¹,
259 $R = 8.314$ J K⁻¹ mol⁻¹, $\rho = 1000$ kg m⁻³, $F = 9.65 \times 10^4$ C mol⁻¹. I and ρ_i are estimated as according to
260 the following composition of the initial WPI powder: protein content of 93.6 wt%, 4.4 wt% moisture,
261 0.3 wt% fat, < 3 wt% lactose and 1.7 wt% ash of which 0.079 wt% Ca²⁺, 0.006 wt% Mg²⁺, 0.037 wt% K⁺,
262 0.767 wt% Na⁺, 0.001 wt% Cl⁻ [30].

263 In Figure 6, Pe as a function of percentage of added salt for all dispersions is shown. In all cases $Pe > 1$,
264 which indicates that particles will accumulate at the surface of the droplet creating a shell [32]. No
265 significant effect of addition of NaCl can be observed. In case of $P_{cap} > P_{buck}$, buckling is expected as
266 soon as the shell becomes elastic, independent of its thickness. Calculated P_{cap} and P_{buck} are shown in
267 Figure 6 and Figure 7 respectively. P_{cap} is inversely proportional to the colloidal size as reported in Eq.
268 4 and it ranges from a minimum of 7×10^6 Pa in case of fractal WPM, to a maximum of 4×10^7 Pa for
269 soluble WPA. P_{buck} , instead, results to be much lower, with a maximum value of 7×10^2 Pa. Hence,
270 buckled/shrivalled morphologies are expected, in agreement to particle morphologies observed in this
271 work (Figure 5). P_{Darcy} is estimated ranging from 3×10^5 to 2×10^7 Pa (Figure 7), in agreement with
272 osmotic pressure values of protein colloids reported in literature [33]. P_{Darcy} confirms solid shell
273 formation and indicates that accumulated colloidal particles are compressed and further aggregated.
274 This pressure difference could be counterbalanced by charge-induced repulsions of colloidal particles,
275 described by the DLVO potential. In this case, DLVO potential results to be always negative for all
276 solutions, and no effect of salt addition is observed, as estimated from Eq. 6. Negative or attractive
277 DLVO potential indicates that colloidal interaction is dominated by van der Waals attraction. The
278 absence of effect of salt addition indicates that the amount of ions already presented in the initial
279 powder is enough to completely screen out the electrostatic repulsion, at the concentrations worked
280 on.

281 The occurrence of a different final particle morphology can now be discussed (Figure 5). In the case of
282 buckling of droplet with a thin elastic layer, an instability with the largest wavelength mode is expected
283 (i.e. bottom right picture in Figure 5). Instead, it is known that if an elastic layer is supported by another
284 elastic layer, the first one being more compressed than the second, an instability may occurs with a
285 wavelength about the thickness of the first layer [34]. Thus, the formation of a shrivalled particle (i.e.
286 bottom left picture in Figure 5) can be explained by assuming the formation of a gel inside the droplets,
287 which is a loose network of proteins. At the surface of the gel, a denser elastic phase is formed, because
288 the Darcy pressure is larger near the surface (P_{Darcy} increases from the centre of the droplets toward
289 its surface). This dense shell is compressed, because of the droplet shrinkage due to evaporation, it
290 cannot sustain the compression and it buckles. However, the inner gel can sustain compression, since
291 it is less dense, and acts like an elastic substrate with a low compression stress. This results in a buckling
292 instability with a wavelength of about the dense layer thickness, leading to a shrivalled dry droplet. At
293 opposite, in the absence of an inner gel, the wavelength selected by buckling is the largest possible
294 and a cup-like shape particle is formed. In this study, a shrivalled morphology is observed only for
295 particles of soluble WPA dispersions. Soluble WPA particles showed the smallest Pe number (Figure 6),
296 indicating that diffusion is more efficient against shell formation when compared to the other protein
297 dispersions investigated. Specifically, diffusion dominates convection at a characteristic length of the
298 order of $D/U = 2R/Pe$, where U is the water velocity at droplet surface, and D the diffusion coefficient
299 given by $D = kT/6\pi\eta a$ (see Eq. 1). Below this length, diffusion dominates, and a homogeneous proteins
300 layer is maintained, which becomes more and more concentrated. However, the layer collapse due to
301 Darcy pressure. For soluble WPA this length is about 10 μ m, instead for fractal WPM it is about 1 μ m.

302 Thus for soluble WPA, the formation of the shell appears at a larger scale. The shell itself exhibits a
303 gradient of elasticity because the Darcy pressure increases from the inner part of the shell to the outer
304 part. A larger shell is more likely to have an uneven density. As explained above, such shells may be
305 able to sustain more compression in their inner part than in its outer part, leading to a shrivelled
306 particle shape. In case of smaller characteristic length, colloidal particles accumulate in a thinner shell,
307 which may collapse more uniformly, inducing a simple folding instability leading to a cup-like shape.
308 This folding to shrivelling transition has been detailed precisely in some model cases [34]. **Obviously,**
309 **the mechanisms suggested here require further studies to be confirmed. But the delicate mechanical**
310 **properties of the gel-like network of proteins, particularly the behaviour under compression, in**
311 **addition to the presence of a gradient of density make a precise modelling of the shells an extremely**
312 **difficult challenge.**

313 **Conclusion**

314 This paper shows the rationalization of morphology development during drying of single droplets of
315 protein suspensions. Non-aggregated and aggregated whey protein dispersions were used as
316 reference systems, with and without salt addition. Morphology development was characterized
317 experimentally by evaluating the evaporation rate, as well as the locking and buckling times, both by
318 macro and microscopy. Results showed no significant difference in terms of evaporation rate, locking
319 and buckling time among the colloidal dispersions and as a function of salt addition. However, a
320 significant characteristic final morphology was observed. Specifically, particle changed from shriveled
321 to cup-like shape at increasing colloidal sizes. Estimation of physical parameters affecting shell
322 formation and mechanical properties anticipated a buckled/shriveled morphology of dry droplets as in
323 agreement to experimental observations. The formation of a solid shell at the particle surface was
324 predicted. In addition, calculation of the DLVO potential showed that colloidal interaction were
325 dominated by van der Waals attraction. Hence, the diverse final morphology was explained by
326 assuming the formation of elastic layers at the particle surface with different compression stress.

327 Finally, this work showed that it is possible to achieve diverse final morphology of particles using same
328 drying conditions and starting from the same type of colloids (WPI) but with different degree of
329 aggregation. In addition, it is possible to predict the final morphology of particle by estimating
330 mechanical properties of the particle shell. **This evidence of applicability of fundamental knowledge,**
331 **in terms of the modelling of the colloidal aspects of dispersion drying, establishes a first base for**
332 **controlling particle formation as well as functionality of food and pharma protein products on a dry**
333 **state.**

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341 **References**

- 342 [1] J. Bouman, P. Venema, R. J. de Vries, E. van der Linden, and M. A. I. Schutyser, "Hole and
343 vacuole formation during drying of sessile whey protein droplets," *Food Res. Int.*, vol. 84, pp.
344 128–135, 2016.
- 345 [2] E. M. Both, A. M. Karlina, R. M. Boom, and M. A. I. Schutyser, "Morphology development
346 during sessile single droplet drying of mixed maltodextrin and whey protein solutions," *Food*
347 *Hydrocoll.*, vol. 75, pp. 202–210, 2018.
- 348 [3] C. Schmidmeier, C. O’Gorman, K. Drapala, D. Waldron, J. O’Mahony, Elucidation of factors
349 responsible for formation of white flecks in reconstituted fat filled milk powders, *Colloids and*
350 *Surfaces A: Physicochemical and Engineering Aspects*, 2019 vol: 575 pp: 245-255
- 351 [4] A. Sharma, A. H. Jana, R. S. Chavan, Functionality of Milk Powders and Milk-Based Powders for
352 End Use Applications—A Review. *Comprehensive Reviews in Food Science and Food Safety*,
353 2012, Vol 11, pp: 518-528
- 354 [5] G. Ronse *et al.*, "Microstructure evolution of micellar casein powder upon ageing:
355 Consequences on rehydration dynamics," *J. Food Eng.*, vol. 206, pp. 57–66, 2017.
- 356 [6] J. Ji, K. Cronin, J. Fitzpatrick, and S. Miao, "Enhanced wetting behaviours of whey protein
357 isolate powder: The different effects of lecithin addition by fluidised bed agglomeration and
358 coating processes," *Food Hydrocoll.*, vol. 71, pp. 94–101, 2017.
- 359 [7] S. V. Crowley, B. Desautel, I. Gazi, A. L. Kelly, T. Huppertz, J. A. O' Mahony, Rehydration
360 characteristics of milk protein concentrate powders, *Journal of Food Engineering*, 2015, vol.
361 149, pp. 05-113.
- 362 [8] C. Sadek, H. Li, P. Schuck, Y. Fallourd, N. Pradeau, C. Le Floch-Fouéré, R. Jeantet, "To What
363 Extent Do Whey and Casein Micelle Proteins Influence the Morphology and Properties of the
364 Resulting Powder?," *Dry. Technol.*, vol. 32, no. 13, pp. 1540–1551, 2014.
- 365 [9] L. Lanotte, F. Boissel, P. Schuck, R. Jeantet, C. Le Floch-Fouéré, Drying-induced mechanisms of
366 skin formation in mixtures of high protein dairy powders, *Colloids and Surfaces A:*
367 *Physicochemical and Engineering Aspects*, 2018 vol: 553 pp: 20-27
- 368 [10] Y. Fang, S. Rogers, C. Selomulya, and X. D. Chen, "Functionality of milk protein concentrate:
369 Effect of spray drying temperature," *Biochem. Eng. J.*, vol. 62, pp. 101–105, 2012.
- 370 [11] E. Panagopoulou, V. Evageliou, N. Kopsahelis, D. Ladakis, A. Koutinas, I. Mandala, Stability of
371 double emulsions with PGPR, bacterial cellulose and whey protein isolate, *Colloids and*
372 *Surfaces A: Physicochemical and Engineering Aspects*, 2017, vol. 522, pp.445-452.
- 373 [12] J. K. Keppler, K. Schwarz, Increasing the emulsifying capacity of whey proteins at acidic pH
374 values through covalent modification with allyl isothiocyanate, *Colloids and Surfaces A:*
375 *Physicochemical and Engineering Aspects*, 2017, vol 522, pp. 514-524.
- 376 [13] M. Chevallier, A. Riaublanc, C. Cauty, P. Hamon, F. Rousseau, J. Thevenot, C. Lopez, T.
377 Croguennec, The repartition of whey protein microgels and caseins between fat droplet
378 surface and the continuous phase governs the heat stability of emulsions, *Colloids and*
379 *Surfaces A: Physicochemical and Engineering Aspects*, 2019, vol. 563, pp. 217-225
- 380 [14] E. Lintingre, F. Lequeux, L. Talini, and N. Tsapis, "Control of particle morphology in the spray
381 drying of colloidal suspensions," *Soft Matter*, vol. 12, no. 36. pp. 7435–7444, 2016.
- 382 [15] G. Gauthier, V. Lazarus, L. Pauchard, Alternating Crack Propagation during Directional Drying,
383 *Langmuir*, 2007, vol 23 (9), pp. 4715-4718

- 385 [16] G. W. Scherer, "Recent progress in drying of gels," *J. Non. Cryst. Solids*, vol. 147–148, no. C,
386 pp. 363–374, 1992.
- 387 [17] P. Atkins and J. de Paula, "Atkins's physical chemistry." Oxford University Press, 2006.
- 388 [18] C. Schmitt, C. Bovay, A.-M. Vuillomenet, M. Rouvet and L. Bovetto, "Influence of protein and
389 mineral composition of whey protein isolates on the formation of microgels upon heat
390 treatment," *Food Hydrocoll.*, vol. 25, no. 4, pp. 558-567, 2011.
- 391 [19] T. Phan-Xuan, D. Durand, T. Nicolai, L. Donato, C. Schmitt and L. Bovetto, "On the crucial
392 importance of the pH for the formation and self-stabilisation of protein microgels and
393 strands," *Langmuir*, vol. 27, no. 24, pp. 15092-15101, 2011.
- 394 [20] G. Unterhaslberger, C. Schmitt, C. Sanchez, C. Appolonia-Nouzille, and A. Raemy, "Heat
395 denaturation and aggregation of β -lactoglobulin enriched WPI in the presence of arginine HCl,
396 NaCl and guanidinium HCl at pH 4.0 and 7.0," *Food Hydrocoll.*, vol. 20, no. 7, pp. 1006–1019,
397 2006.
- 398 [21] S. W. Provencher and P. Štěpánek, "Global analysis of dynamic light scattering autocorrelation
399 functions," *Part. Part. Syst. Charact.*, vol. 13, no. 5, pp. 291–294, 1996.
- 400 [22] K. Kolwas, G. Derkachov, M. Zientara, M. Kolwas, and D. Jakubczyk, "Evaporation of Micro-
401 Droplets: the 'Radius-Square-Law' Revisited," *Acta Phys. Pol. A*, vol. 122, no. 4, pp. 709–716,
402 2016.
- 403 [23] K. N. Ryan, B. Vardhanabhuti, D. P. Jaramillo, J. H. van Zanten, J. N. Coupland, and E. A.
404 Foegeding, "Stability and mechanism of whey protein soluble aggregates thermally treated
405 with salts," *Food Hydrocoll.*, vol. 27, no. 2, pp. 411–420, 2012.
- 406 [24] C. Schmitt *et al.*, "Internal structure and colloidal behaviour of covalent whey protein
407 microgels obtained by heat treatment," *Soft Matter*, vol. 6, no. 19, pp. 4876–4884, 2010.
- 408 [25] T. Nicolai, M. Britten, and C. Schmitt, " β -Lactoglobulin and WPI aggregates: Formation,
409 structure and applications," *Food Hydrocoll.*, vol. 25, no. 8, pp. 1945–1962, 2011.
- 410 [26] T. Nicolai, D. Durand, A. Riaublanc, N. Mahmoudi, and S. Mehalebi, "Light-Scattering Study of
411 the Structure of Aggregates and Gels Formed by Heat-Denatured Whey Protein Isolate and β -
412 Lactoglobulin at Neutral pH," *J. Agric. Food Chem.*, vol. 55, no. 8, pp. 3104–3111, 2007.
- 413 [27] S. Mehalebi, T. Nicolai, and D. Durand, "Light scattering study of heat-denatured globular
414 protein aggregates," *Int. J. Biol. Macromol.*, vol. 43, no. 2, pp. 129–135, 2008.
- 415 [28] J. M. Aguilera and P. Baffico, "Structure-mechanical properties of heat-induced whey
416 protein/cassava starch gels," *J. Food Sci.*, vol. 62, no. 5, 1997.
- 417 [29] D. A. Weitz *et al.*, "Onset of Buckling in Drying Droplets of Colloidal Suspensions," *Phys. Rev.*
418 *Lett.*, vol. 94, no. 1, 2005.
- 419 [30] C. Schmitt, C. Bovay, M. Rouvet, S. Shojaei-Rami, and E. Kolodziejczyk, "Whey protein soluble
420 348 aggregates from heating with NaCl: Physicochemical, interfacial, and foaming properties,"
421 349 *Langmuir*, vol. 23, no. 8, pp. 4155–4166, 2007.
- 422 [31] S. Torquato, T. M. Truskett, and P. G. Debenedetti, "Is random close packing of spheres well
423 defined?," *Phys. Rev. Lett.*, vol. 84, no. 10, pp. 2064–2067, 2000.
- 424 [32] R. Vehring, "Pharmaceutical particle engineering via spray drying," *Pharm. Res.*, vol. 25, no. 5,
425 pp. 999–1022, 2008.

- 426 [33] A. Bouchoux, P. Qu, P. Bacchin, and G. Gésan-Guiziu, "A general approach for predicting the
427 filtration of soft and permeable colloids: The milk example," *Langmuir*, vol. 30, no. 1, pp. 22–
428 34, 2014.
- 429 [34] F. Brau, P. Damman, H. Diamant, and T. A. Witten, "Wrinkle to fold transition: Influence of the
430 substrate response," *Soft Matter*, vol. 9, no. 34. pp. 8177–8186, 2013.
- 431

Table 1. Composition and pH of colloidal dispersions

Whey protein aggregates	Solid content of WPI dispersions (wt%)	pH-adjustment
Whey protein microgels (WPM)	4.0	5.9
Fractal WPM	7.0	5.9
Soluble whey protein aggregates (WPA)	7.0	6.9

Table 2. Hydrodynamic diameter and polydispersity of colloidal dispersions

Colloidal dispersions	Added NaCl [wt% db]	Hydrodynamic diameter [nm]		Polydispersity index	
		Average	SD*	Average	SD*
Filtered WPI	0	11.73	0.05	0.52	0.002
WPI	0	182	2.89	0.32	0.026
Soluble WPA	0	49.2	0.23	0.28	0.021
	1	49.5	0.88	0.29	0.019
	3	54.9	0.75	0.34	0.016
	6	49.2	0.42	0.28	0.015
WPM	0	230	3.00	0.18	0.021
	1	229	0.47	0.19	0.006
	3	229	1.28	0.17	0.016
	6	227	1.35	0.17	0.008
Fractal WPM	0	291	6.89	0.35	0.026
	1	290	4.31	0.34	0.035
	3	293	2.65	0.35	0.040
	6	289	1.4	0.36	0.008

*SD = standard deviation

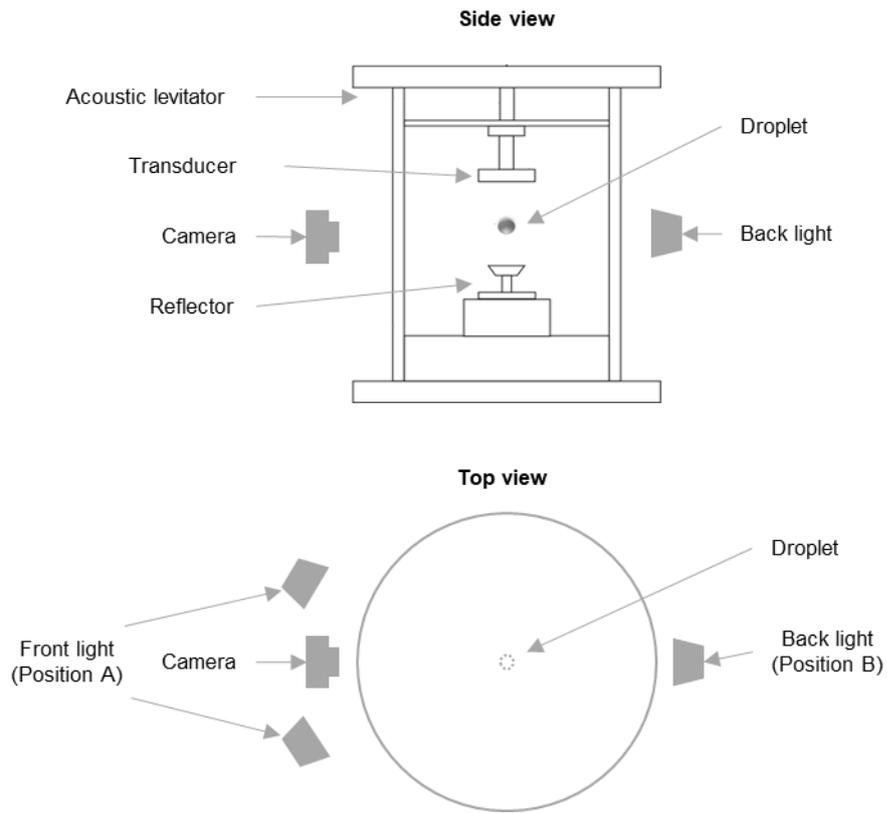


Figure 1. Sketch of the single-drop drying-kinetics device

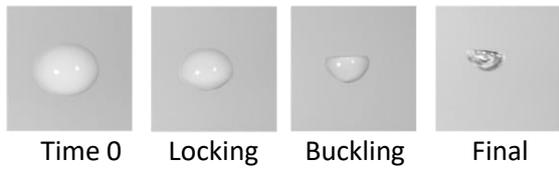


Figure 2. Example images corresponding to characteristic times of morphology development of a single droplet

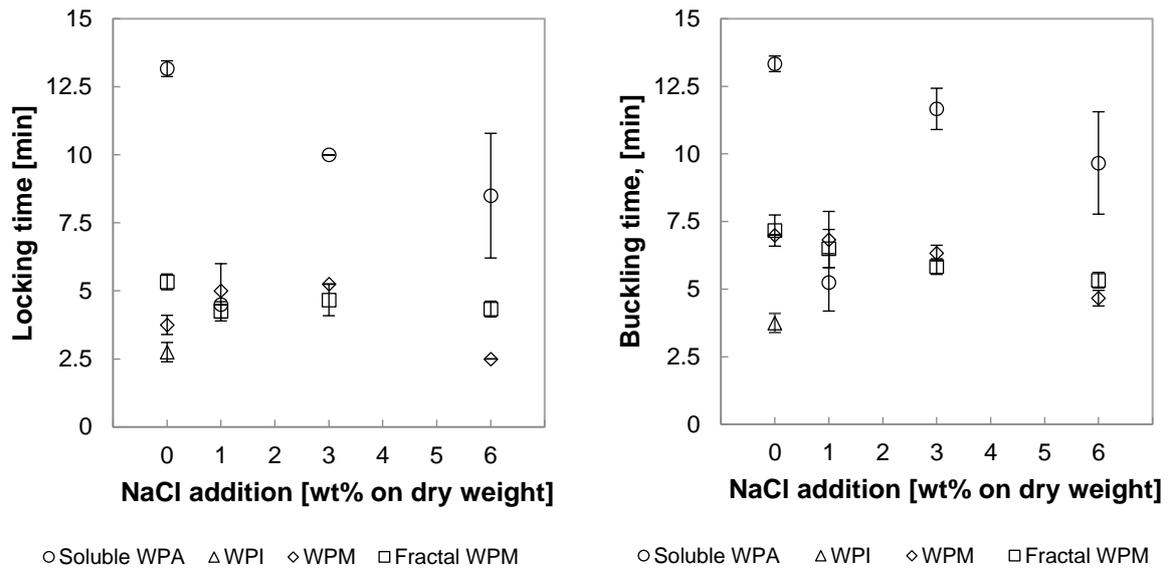


Figure 4. Locking time (left) and buckling time (right) as a function of NaCl addition for soluble WPA, WPI, WPM and fractal WPM droplets.

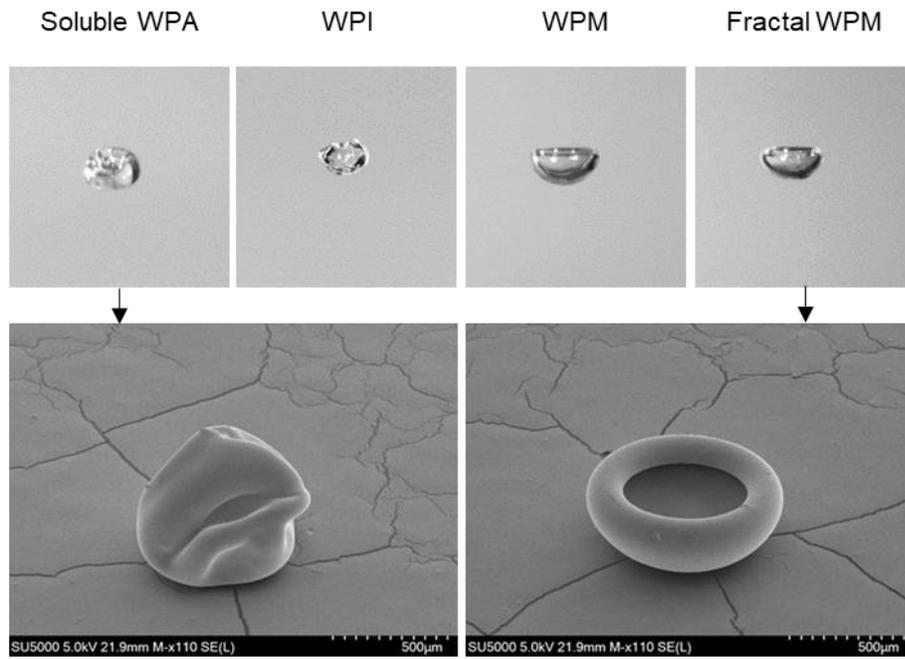


Figure 5. Top pictures: final morphology of soluble WPA, WPI, WPM and fractal WPM droplets captured using a macro camera. Bottom pictures: final morphology of soluble WPA (left) and fractal WPM (right) observed by SEM.

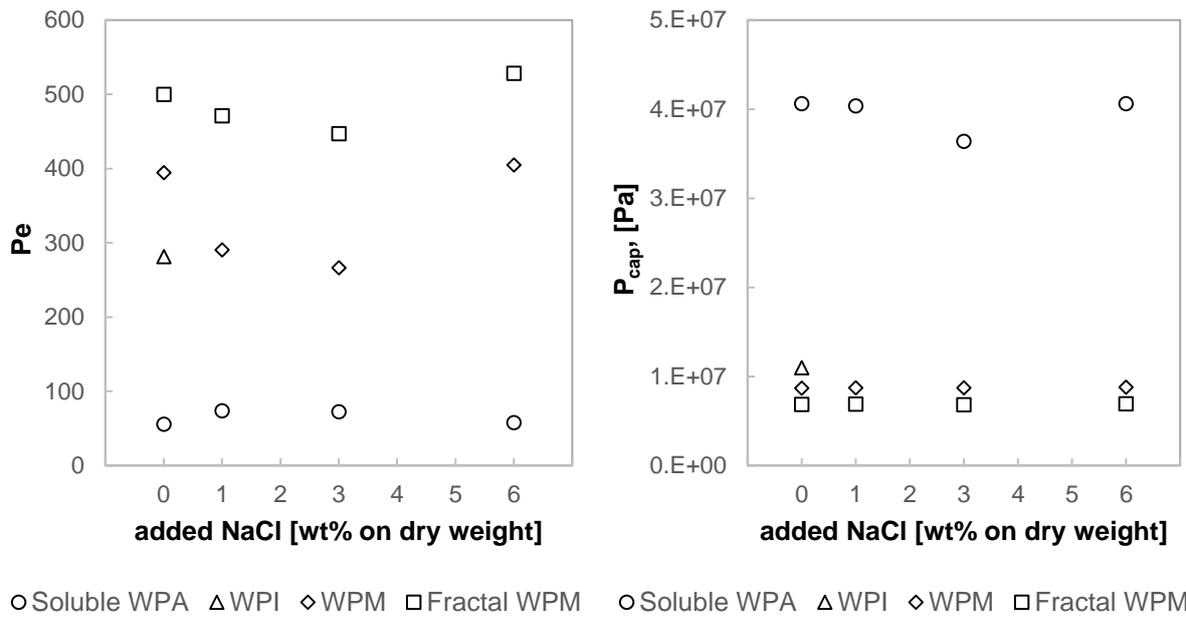


Figure 6. Péclet dimensionless number (left) and Capillary pressure (right) as a function of NaCl addition for soluble WPA, WPI, WPM and fractal WPM droplets.

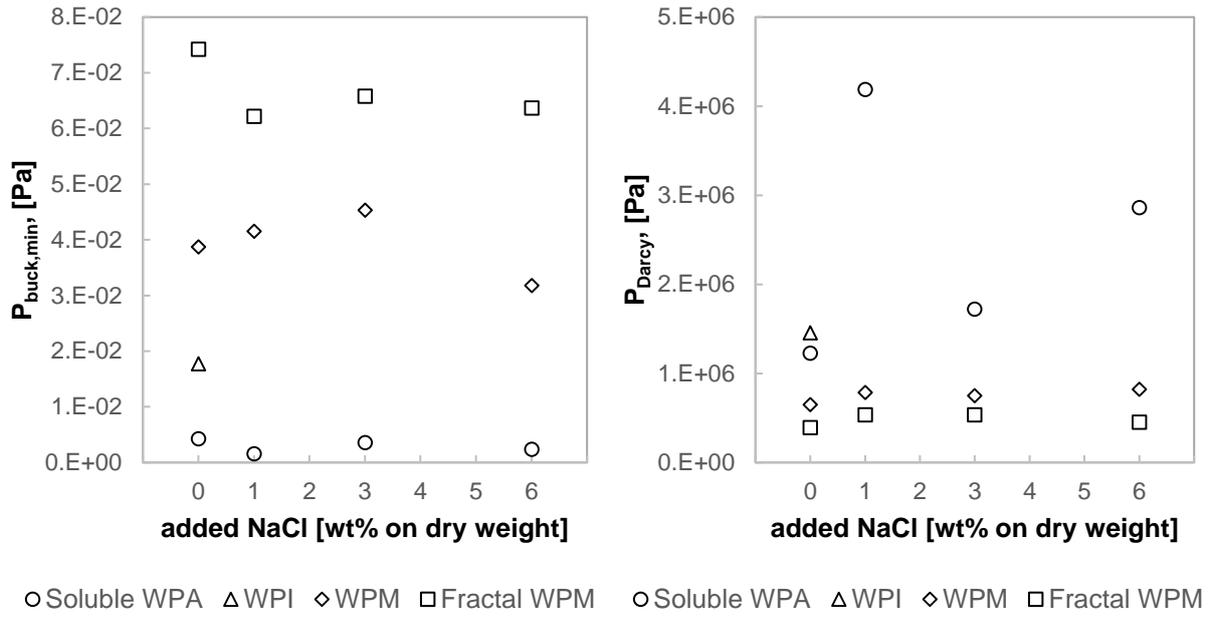


Figure 7. Minimum buckling pressure (left) and Darcy's pressure (right) as a function of NaCl addition for soluble WPA, WPI, WPM and fractal WPM droplets.