

In-vitro oral digestion of microfluidically produced monodispersed W/O/W food emulsions loaded with concentrated sucrose solution designed to enhance sweetness perception

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1 ***In-vitro* oral digestion of microfluidically produced monodispersed W/O/W food**
2 **emulsions loaded with concentrated sucrose solution designed to enhance sweetness**
3 **perception**
4

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11

12 **Abstract**

13

14 Monodispersed W₁/O/W₂ emulsions consisting of sunflower oil droplets containing a single
15 large internal droplet or numerous small internal droplets of concentrated sucrose solution
16 were prepared by microfluidic emulsification. The external droplet interface was stabilized by
17 waxy rice starch, which hydrolyzes during oral processing thereby releasing the encapsulated
18 sucrose solution to the proximity of taste receptors imparting a higher sweetness perception
19 compared to adding the same amount of sugar to the bulk phase. The sucrose release was
20 tracked by adding NaCl to the internal phase as a conductivity tracer. Core/shell droplets
21 containing 50 wt% sucrose and 1.5 wt% NaCl in the internal phase, 1.40–2.86 wt%
22 polyglycerol polyricinoleate (PGPR) in the middle phase, and 4 wt% gelatinized waxy rice
23 starch in the external phase were produced with 100% encapsulation efficiency and showed
24 stability against coalescence for at least two months, because the gelatinized starch acted as a
25 highly efficient Pickering stabilizer. The sucrose release from the inner droplets during *in-*
26 *vitro* oral processing at 37°C for 30 s with 50 U/mL α-amylase increased from 16% to 49%
27 when the PGPR concentration in the oil phase was reduced from 2.86 wt% to 0.7 wt%.
28 Core/shell droplets were less stable during storage when the surface-active molecularly

29 dissolved octenyl succinic anhydride (OSA) modified starch was selected as stabilizer
30 although the oil droplets were smaller due to the lower interfacial tension at the external
31 interface. $W_1/O/W_2$ emulsion consisting of numerous internal droplets coalesced during
32 storage in one day and released 91% of sucrose during *in-vitro* oral processing.

33

34 **Keywords:** Encapsulation, Microfluidics, W/O/W emulsion, *In-vitro* oral digestion, Food
35 emulsions, Sweetness perception enhancement.

36

37 **1. Introduction**

38

39 A multiple emulsion consists of internal droplets enclosed within larger droplets, which
40 are themselves dispersed in an external continuous liquid phase. Multiple emulsions have
41 been used in a variety of applications in food, pharmaceutical, and cosmetic industries
42 (Benichou, et al. 2004; Vladisavljević et al. 2017; Kukizaki & Goto 2007; Muschiolik 2007).
43 They offer food processors a means to produce reduced-fat food products by replacing part of
44 the oil with inner water droplets while maintaining a similar surface area of oil droplets
45 (Muschiolik & Dickinson 2017) and to produce reduced-sodium foods without compromising
46 the saltiness perception by creating high salt gradients between inner and outer aqueous phase
47 (Chiu et al. 2015). Multiple emulsions can also be used to improve sensory characteristics of
48 foods by masking unpleasant taste and flavor, to protect sensitive ingredients, e.g. vitamins,
49 antioxidants, probiotics, flavors, and minerals from environmental stresses such as oxygen,
50 light, and pH and ionic strength changes (Jiménez-Colmenero 2013; Muschiolik 2007), and
51 to achieve controlled release of active ingredients during oral processing and digestion
52 (Benichou et al. 2004; Dickinson 2011; McClements et al. 2007; Muschiolik 2007; Chiu et al.
53 2015; Jiménez-Colmenero 2013; Muschiolik & Dickinson 2017; Norton & Norton 2010).

54 Multiple emulsions are usually produced by a two-step emulsification procedure where
55 a single emulsion (O_1/W or W_1/O) is prepared and then re-emulsified in the second
56 immiscible liquid (O_2 or W_2) to prepare a $O_1/W/O_2$ or $W_1/O/W_2$ emulsion, respectively
57 (McClements et al. 2007; Lamba et al. 2015). The first emulsification step is typically done
58 using high energy inputs to prepare fine droplets while the second step is carried out at
59 relatively low shear rates to prevent expulsion of internal droplets into the continuous phase.
60 In conventional devices such as rotor-stator mixers and high-pressure homogenizers, the risk
61 of losing the internal phase during secondary emulsification increases with increasing shear

62 rate (Florence & Whitehill, 1982). The highest encapsulation efficiency can be achieved at
63 the lowest share rate, but it usually leads to highly polydisperse outer drops. Another problem
64 with traditional devices is that the mean droplet size (D_p) is difficult to control. Many
65 properties of multiple emulsions such as their physicochemical stability, optical properties,
66 rheological behavior, *in-vitro* digestibility, and release profile of encapsulated nutrients
67 depend on D_p (McClements, 2005). For fundamental investigations of the behavior of
68 multiple emulsions, it is highly desirable to prepare multiple emulsions with tightly
69 controlled droplet size that can easily be varied.

70 Microfluidic emulsification is a new strategy of generating multiple emulsions, which
71 offers unprecedented control over the size of both internal and external droplets and ~100%
72 encapsulation efficiency within internal droplets (Vladisavljević et al. 2012; Al Nuumani et
73 al. 2018). Microfluidic devices allow facile control over the number of internal droplets
74 (Nabavi et al. 2017) and enable to enclose internal droplets of different solutions within the
75 same outer drops (Sun et al. 2010). Furthermore, they provide an opportunity to achieve
76 complex droplet architectures, such as bifacial (Janus) and triphasic (ternary) drops, and
77 nested drops consisting of multiple concentric layers around each core (Vladisavljević et al.
78 2017). The majority of microfluidic devices make multiple emulsions using two sequential
79 pinch-off events, usually in two consecutive T-junctions of alternating wettability (Okushima
80 et al. 2004), which typically results in core/shell drops with thick shells (Abate et al. 2011).

81 There is a strong need to reduce fat, sugar and salt intake in our diet due to the health
82 issues associated with their consumption (Knüppel *et al.*, 2017). The use of multiple
83 emulsions is a promising strategy to reduce the content of these potentially harmful
84 ingredients in liquid and semi-liquid food products without compromising their taste. Two
85 alternative approaches that can be used to enhance sweetness or saltiness perception in food
86 products using multiple emulsions are: (a) to increase sugar or salt concentration in the
87 continuous aqueous phase while keeping the tastant concentration within internal droplets at
88 low levels and promoting in-mouth stability of multiple emulsion during oral processing
89 (Buyukkestelli et al. 2019; Lad et al. 2012), and (b) to keep sugar or salt concentration in the
90 continuous aqueous phase at low levels while increasing the tastant content within internal
91 droplets to very high levels and promoting destabilization of the multiple emulsion during
92 oral processing (Chiu et al. 2015). In the former case, the tastant is delivered to the taste
93 receptors through the bulk of the food and taste intensity correlates to tastant concentration in
94 the continuous phase. The role of the multiple emulsion here is to concentrate the tastant in

95 the continuous phase, thereby enhancing its perception, while minimizing the amount of
96 added lipid phase. In the latter approach, a water-soluble surfactant added to the continuous
97 phase is designed to quickly break down when brought into contact with saliva, thus releasing
98 high concentrations of tastant from the internal droplets in the proximity of taste receptors
99 located on the tongue. Small pockets of concentrated tastant solution impart a higher
100 perceived taste intensity compared to the case where all of the tastants are present in the
101 external phase at moderate or low concentrations (Burseg et al. 2012).

102 The main objective of this study was to evaluate the feasibility of the sweetness
103 enhancement technology based on burst release of sugar from internal droplets using multiple
104 emulsions with a single large core droplet or numerous tiny internal droplets. For the first
105 time, microfluidic devices composed of coaxial assemblies of borosilicate glass capillaries
106 were used to encapsulate a concentrated sugar solution within starch-stabilized oil droplets.
107 Core/shell droplets were produced using one dripping instability, which allows the shell
108 thickness to be easily adjusted over a wide range (Nabavi et al. 2015). Borosilicate glass is a
109 food-safe and inert material with superior optical transparency, which enables to monitor and
110 control the drop generation process in real time.

111 Droplets were coated with either gelatinized waxy rice starch or molecularly dispersed,
112 surface active, octenyl succinic anhydride (OSA) modified starch. The gelatinized waxy rice
113 starch, which was not surface active, was adsorbed to the external water-oil interface (O/W₂)
114 as aggregated, insoluble starch, thus acting as Pickering stabilizer. Waxy rice starch was
115 selected to reduce the complexity of the system, because waxy starch varieties contain almost
116 100% amylopectin, and not a mixture of amylopectin and amylose. Utilizing a starch
117 emulsifier, the emulsion destabilizes during oral processing due to the mechanical actions of
118 tongue and teeth combined with the enzymatic digestion of the starch, and the encapsulated
119 sugar solution is released into the external phase. The sugar encapsulation efficiency, storage
120 stability, and sugar release during *in-vitro* digestion were compared for emulsions with
121 different formulations and droplet morphologies.

122

123 **2. Experimental**

124

125 **2.1 Chemicals**

126

127 A clean-label native waxy rice starch (Synergie Nutrylon) obtained from Ulrick and
128 Short Ltd (Pontefract, UK) and octenyl succinic anhydride (OSA) modified waxy maize

129 starch (N-Creamer[®] 46, NC46, Univar, UK) were used as hydrophilic emulsifiers. Sunflower
130 oil, table salt and sugar were purchased from a local supermarket (Tesco, UK). NaCl was
131 added to the inner phase to track the release of aqueous phase from the oil phase.
132 Polyglycerol polyricinoleate (GRINDSTED[®] PGPR, DuPont, Kettering, UK) was used as the
133 lipophilic emulsifier. α -Amylase from porcine pancreas with an enzymatic activity of 10
134 U/mg and sodium azide (antimicrobial agent) were supplied by Sigma Aldrich (Gillingham,
135 UK). Sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate obtained
136 from Fisher Scientific were used to prepare phosphate buffer solution. All aqueous solutions
137 were prepared using milli-Q water with a conductivity below 0.5 μ S/cm.
138 Octadecyltrimethoxysilane (OTMS) and 2-
139 [methoxy(polyethyleneoxy)propyl]trimethoxysilane (TMS-PEG) supplied by Fluorochem
140 (Hadfield, UK) were used for surface treatment of glass capillaries.

141

142 **2.2 Fabrication of microfluidic devices**

143

144 A 2-phase glass device was used to produce a $(W_1/O)/W_2$ emulsion composed of a
145 multitude of inner droplets. A 3-phase glass device was used to produce a $W_1/O/W_2$ emulsion
146 containing only one large inner drop. A round capillary (0.58 mm inner diameter and 1 mm
147 outer diameter, Intracel, UK) was pulled using a Flaming/Brown micropipette puller (P-97,
148 Sutter Instrument, UK) until it broke apart into two halves with tapered tips. Abrasive paper
149 was used to polish both tips to the desired orifice size, which was checked using a microforge
150 microscope (Narishige model MF-830). The capillary tips were cleaned with compressed air,
151 washed with Milli-Q water and treated with TMS-PEG or OTMS to render them hydrophilic
152 and hydrophobic, respectively. A square capillary with an inner width of 1.05 ± 0.1 mm (AIT
153 Glass, Rockaway, NJ) was attached to the microscope slide using a two-part epoxy glue. 2-
154 phase devices were assembled by inserting one round capillary with a hydrophilic tip into the
155 square capillary. To assemble a 3-phase device, two round capillaries were inserted from
156 each end of the square capillary at a desired distance from each other, which was checked
157 using an inverted microscope onto which the device was placed. The tip of the injection
158 capillary was made hydrophobic, while the collection capillary had a hydrophilic tip.
159 Hypodermic needles with plastic hub were attached to the capillaries to introduce different
160 liquids into the device. Each device was left for 24 h to ensure the glue was fully cured.

161 These devices were recently replaced by more reproducible glue-free devices assembled
162 using 3D printed capillary holders (Bandulasena et al. 2019).

163

164 **2.3 Emulsion formation**

165

Fig. 1

166 A schematic of the experimental setup is shown in Figure 1. Gastight glass syringes
167 with Luer-lock fitting (VWR Catalyst Company, UK) were loaded with feed solutions,
168 installed on Harvard Apparatus 11 Elite syringe pumps and delivered through polyethylene
169 medical tubing (0.86 mm I.D., 1.52 mm O.D., Fisher Scientific, UK) at controlled flow rates.
170 The drop generation process, which depended on stream flow rates and device geometry, was
171 observed and recorded using a GT Vision inverted microscope and Phantom V9.0 high-speed
172 camera. ImageJ v.1.44 software (Wayne Rasband, National Institute of Health) was used to
173 measure the average droplet diameter and frequency of droplet formation from the recorded
174 pictures captured with a resolution of 768×576 pixels at 2000 frames per second.

175 Multiple emulsion droplets with one large internal droplet were prepared upon the
176 break-up of a compound jet composed of two coflowing liquids (the inner phase and the
177 middle phase) in a 3-phase device. The inner phase was 1.5 wt% NaCl and 50 wt% sucrose in
178 water, the outer phase was a mixture of 25 wt% sucrose and 4 wt% gelatinized waxy rice
179 starch in water, and the middle phase was 2.86 wt%, 1.4 wt% or 0.7 wt% PGPR in sunflower
180 oil. Starch was gelatinized by mixing a suspension of starch in water with a high shear
181 overhead mixer (LM5 fitted with emulsor screen, Silverson, Chesham, UK) at 8,000 rpm for
182 5 min. During this process the temperature increased from ambient to 60-70 °C (Kasprzak et
183 al. 2018).

184 Multiple emulsion droplets containing numerous small internal droplets were produced
185 upon the breakup of the pre-formed W_1/O emulsion in a 2-phase device. The primary W_1/O
186 emulsion was prepared using a high shear overhead mixer (LM5 fitted with emulsor screen,
187 Chesham, UK). First, 2 g of PGPR was mixed with 68 g of sunflower oil for 2 min at 8,000
188 rpm to prepare the middle phase, followed by addition of 30 g of the inner phase containing
189 1.5 wt% NaCl, 50 wt% sucrose and 0.02 wt% NaN_3 and shearing for 5 min at 8,000 rpm.

190

191 **2.4 Encapsulation efficiency of sucrose, *in-vitro* digestion and emulsion stability**

192

193 Encapsulation efficiency of sugar in freshly produced multiple emulsions was estimated
194 by incorporating NaCl as a conductometric tracer in the internal droplets. For *in-vitro*

195 digestion, 9 g of the prepared emulsion were transferred into a 50 mL glass beaker containing
 196 a magnetic stirring bar followed by the addition of 9 mL of porcine amylase solution in
 197 phosphate buffer (100 U/mL, 0.01 M) resulting in the final enzyme activity of 50 U/mL
 198 mixture. The mixture was stirred at 37°C and 500 rpm for 30 s to mimic food processing in
 199 the oral cavity. The amount of internal phase released was determined based on the electric
 200 conductivity of the mixture before and after digestion. The calibration graphs were
 201 constructed by measuring the conductivity of O/W emulsions with known amounts of salt in
 202 the external phase prepared with the same overall composition as the investigated emulsion
 203 samples. The detailed calibration procedure is provided in the supplementary information.
 204 The conductivity was measured using a Mettler Toledo Model inLab[®] 710 conductivity meter
 205 with a measuring range of 0.01–500 mS/cm, connected with a 4-pole platinum conductivity
 206 cell with a chemical resistant glass body. The stability of prepared emulsions against
 207 coalescence was estimated under stagnant conditions after 1 day, 7 days, and 60 days. The
 208 formation of a yellowish oil layer on top of the cream phase and clearly visible large oil
 209 droplets were taken as signs of coalescence.

210

211 3. Results and Discussion

212 3.1 Production of W₁/O/W₂ emulsion

213 Core/shell droplets with tunable size and shell thickness were produced by controlling
 214 the flow rates of the inner, middle and outer phase, Q_1 , Q_2 , and Q_3 (Figure 2), most notably
 215 the flow rate ratio Q_2/Q_1 . The dimensionless parameter ζ can be used to predict the droplet
 216 generation regime (Nabavi et al. 2017b):

$$217 \quad \zeta = \left[\frac{Ca_1^{0.25}}{Ca_2^{0.57} Ca_3^{1.12}} \right] \left[\frac{D_{orif}}{D_N} \right] \quad (1)$$

218 where D_{orif} is the orifice diameter of the collection capillary, D_N is the internal nozzle
 219 diameter of the injection capillary (see Fig. 1), whereas Ca_1 , Ca_2 , and Ca_3 are the capillary
 220 numbers of the inner, middle and outer phase, respectively, given by (Nabavi et al. 2015):

$$221 \quad Ca_1 = \frac{\mu_1 V_1}{\sigma_{12}} \quad Ca_2 = \frac{\mu_2 V_2}{\sigma_{23}} \quad Ca_3 = \frac{\mu_3 V_3}{\sigma_{23}} \quad (2)$$

222 where $V_1 = 4Q_1/(\pi D_N^2)$, $V_2 = Q_2/(D_{co}^2 - \pi D_{ci}^2/4)$, and $V_3 = Q_3/(D_{co}^2 - \pi D_{ci}^2/4)$ are the
 223 characteristic velocities of the inner, middle and outer phase, D_{ci} is the internal height (or
 224 width) of the square capillary, and D_{co} is the outer diameter of the inner capillary.

225 At $\log \zeta > 5.7$, a multiple emulsion was formed in the dripping regime (Fig. 2a-c),
 226 which resulted in monodisperse droplets, while jetting occurred at $\log \zeta < 5.7$ (Fig. 2d).
 227 Based on Eq. (1), jetting occurs at very low Q_1 value or high Q_2 and Q_3 values. The reason
 228 for jetting in Fig. 2d is the very low Q_1 value, resulting in large polydisperse droplets.
 229 According to the National Institute of Standards and Technology (NIST), “a particle size
 230 distribution may be considered monodisperse if at least 90% of the distribution lies within 5%
 231 of the median size”. For a normal particle size distribution, it implies that droplets are
 232 monodisperse if $CV < 3\%$ (Vladislavljević et al. 2018), where $CV = D_2/\sigma$ is the coefficient of
 233 variation (D_2 is the average diameter of external droplets and σ is the standard deviation of
 234 their size).

235 Fig. 2

236 At $Q_2/Q_1 = 3.3$ (Fig. 2a), the middle fluid jet was shorter than in Fig. 2d with a shell
 237 thickness (t_s) of 64 μm . With further decrease in Q_2/Q_1 below unity (0.7; Fig. 2b-c), droplets
 238 with thin shells ($t_s = 11$ and 27 μm) were formed close to the orifice. Therefore, the jet
 239 break-up length, droplet size, and shell thickness can be all controlled over a wide range by
 240 adjusting fluid flow rates.

241 The effect of fluid flow rates on the droplet diameters, D_1 and D_2 , and shell thickness,
 242 t_s in the dripping regime is shown in Fig. 3.

243 Fig. 3

244 As shown in Fig. 3a, an increase in Q_1 resulted in a small increase in D_1 , due to higher
 245 inflow of the inner phase during jet pinch-off. However, Q_1 had no impact on the shear stress
 246 at the external oil-water interface and D_2 was unaffected by Q_1 . Both trends resulted in a
 247 decrease in the shell thickness, t_s . The material balance equation can be written in the
 248 following form: $Q_1 + Q_2 = (\pi D_2^3/6)f$, where f is the drop generation frequency. Therefore:

$$249 \quad f = 6\pi^{-1}D_2^{-3}(Q_1 + Q_2) \quad (3)$$

250 Thus, at constant D_2 and Q_2 , an increase in Q_1 led to an increase in f , as found in Fig. 3a.

251 As shown in Fig. 3b, increasing Q_2 at constant Q_1 and Q_3 led to an increase in D_2 , due
 252 to smaller shear stress at the external interface, while D_1 remained constant and thus, the oil
 253 shell became thicker. According to Eq. (3), f increased with increasing Q_2 .

254 Fig. 3c shows that increasing Q_3 reduced both D_1 and D_2 but by the same extent and the
 255 shell thickness remained constant. A decrease in D_1 and D_2 with an increase in Q_3 can be
 256 attributed to the increase in viscous stress exerted by the outer fluid to compound jet, which
 257 led to more frequent drop pinch-off and smaller droplets.

258 The droplet generation behavior was stable over at least five hours, as shown in Fig. 3d.
259 There was no noticeable change in the diameter of the produced droplets and the frequency of
260 droplet generation. No wetting of the capillary walls was observed over 5 h, indicating that
261 the surface treatment of glass wall by organosilicon compounds was robust.

262 The collected core/shell droplets are shown in Figure 4. In Fig. 4a, one small satellite
263 droplet of internal aqueous phase was enclosed within each oil droplet in addition to the main
264 aqueous drop. As the jet starts to pinch off it creates a neck between the jet and the
265 developing drop. Satellite droplets are formed because the neck breaks up at multiple
266 locations during jet pinch-off (Nabavi et al. 2015b).

267 Fig. 4

268 The shell thickness depends on the middle phase to inner phase flow rate ratio, Q_2/Q_1 ,
269 during droplet generation and the size of inner droplets, D_1 :

$$270 \quad t_s = \frac{D_1}{2} \left\{ \left[1 + \left(\frac{Q_2}{Q_1} \right)^{1/3} \right] - 1 \right\} \quad (4)$$

271 In Fig. 4a, Q_2/Q_1 was 12.5 leading to thicker shell than in Figs. 4b, when Q_2/Q_1 was 3.
272 Under similar flow rates, the droplets stabilized by OSA starch (Fig. 4c) were noticeably
273 smaller than the droplets stabilized by waxy rice starch (Fig. 4b), which can be attributed to
274 the amphiphilic and thereby the interfacial tension reducing character of OSA-modified
275 starch, due to the introduction of hydrophobic alkenyl groups of OSA, while the waxy rice
276 starch was heat treated and does not have the ability to reduce the interfacial tension
277 (Kasprzak et al. 2018). The higher interfacial tension at the external oil-water led to the
278 higher ability of the compound jet to resist break up during pinch-off resulting in larger
279 droplets (Nabavi et al. 2015).

280

281 **3.2 Encapsulation efficiency and storage stability of emulsions**

282 Emulsion stability is a critical factor in the food industry as most of the food emulsions
283 are stored after production. In this case, emulsifiers should stabilize both oil-water interfaces
284 during storage but should impart instability during oral processing to provide a burst release
285 of sugar in the vicinity of taste buds. Core/shell droplets stabilized with 2.86 wt% PGPR in
286 the middle phase and 4 wt% OSA starch in the outer phase coalesced after ~1 h. However,
287 core/shell droplets containing 1.4 wt% or 2.86 wt% PGPR and 4 wt% gelatinized starch were
288 stable over 60 days (Table 1). The droplet stability in the presence of gelatinized starch was
289 achieved in spite of an osmotic gradient of ~513 mOsmol induced by the salt addition to the

290 internal droplets, which is more than 2.5 times higher than the recommended osmotic
291 gradient (180–200 mOsmol) for obtaining stable multiple emulsions (Muschiolik et al. 2006).

292 Table 1

293 High stability of core/shell droplets in the presence of gelatinized starch can be
294 explained by high degree of association of starch molecules after thermal treatment due to
295 realignment of amylose and amylopectin. These large hydrogen-bonded aggregates with a
296 molecular weight M_w of 91.9 MDa (Kasprzak et al. 2018) adhered to the external droplet
297 interface forming a thick interfacial layer that imparted a long-term stability to the droplets.
298 On the other hand, after esterification of starch with OSA, steric hindrance effects imposed
299 by bulky OSA groups prevent the alignment of molecular chains of starch resulting in a small
300 degree of association between starch molecules and a M_w of 0.47 MDa (Kasprzak et al.
301 2018). As a result, OSA-waxy maize starch (NC46) can easily diffuse to the external
302 interface due to its small amphiphilic molecules forming a thin interfacial layer. Interestingly,
303 4 wt% OSA starch was able to impart a long-term stability to an O/W emulsion with a droplet
304 size of several microns (Kasprzak et al. 2018).

305 To estimate the encapsulation efficiency of sucrose, a freshly prepared emulsion was
306 gently mixed and its electric conductivity was measured and used to estimate the amount of
307 salt in the external aqueous phase. The encapsulation efficiency, EE was calculated as:

$$308 \quad EE = (m_1 - m_2)/m_1 \quad (5)$$

309 where m_1 is the mass of NaCl added to the inner phase and m_2 is the mass of NaCl in the
310 external phase after emulsion collection. The EE was ~100% for all of the formulations
311 except when the content of PGPR in the middle phase was 0.7 wt% (Table 1) suggesting that
312 0.7 wt% PGPR was not enough to stabilize the droplets and thus 30 % of the salt was
313 released during emulsion preparation. This finding agreed with the stability test results, since
314 coalescence of oil droplets was observed on day 1. High encapsulation efficiencies of small
315 molecules within core-shell droplets were observed in many microfluidic devices (Li et al.
316 2018).

317 Typical instability mechanisms and release pathways involved in a W/O/W emulsion
318 are the expulsion of internal droplets into the external phase, the coalescence of internal
319 droplets before expulsion from multiple emulsion drops, and the shrinkage or swelling of
320 internal droplets due to osmotic pressure gradient across the oil phase (Benichou & Aserin
321 2008; Florence & Whitehill 1981). In this case, the expulsion of internal droplets was most
322 likely responsible for the low EE value at 0.7 wt% PGPR. Therefore, the PGPR content in the

323 oil phase should be at least 1.4 wt% to impart droplet stability during microfluidic
324 emulsification and subsequent storage.

325 Multiple emulsion droplets containing numerous small internal droplets with a mean
326 diameter of about 4 μm showed 100% *EE* during microfluidic emulsification but very poor
327 storage stability (Table 1). In core/shell droplets, swelling of internal droplets was suppressed
328 by the relatively thick oil layer around the internal water droplets. The diffusion of water is
329 inversely proportional to the shell thickness, which was 42–58 μm for the droplets in Table 1.
330 In multiple emulsion droplets with numerous small internal droplets, some internal droplets
331 were located very close to the external interface, which led to fast swelling of internal
332 droplets and their burst from the oil phase. $(W_1/O)/W_2$ emulsions containing 1–2 wt% PGPR
333 and 1–4 wt% gelatinized waxy rice starch prepared by Kasprzak et al. (2019) using a high-
334 shear mixer showed a high long-term stability, which means that stability of multiple
335 emulsions during storage under stagnant conditions strongly depends on droplet size,
336 probably due to different creaming rates.

337

338 **3.3 Release of sucrose during *in-vitro* digestion**

339 To study the release of sucrose during digestion, an *in-vitro* oral cavity model was used.
340 Core/shell droplets stabilized with OSA starch burst shortly after fabrication and were not
341 used here. In addition, OSA starch is less accessible to salivary amylase compared to native
342 starch and shows a lower digestion kinetics (He et al. 2008; Lin et al. 2018).

343 The sucrose release was calculated as:

$$344 \quad \text{sucrose release } (R) = (m_2 - m_3)/(m_1 - m_2) \quad (6)$$

345 where m_3 is the mass of NaCl in the internal droplets after digestion.

346 The highest release of sucrose was observed in $(W_1/O)/W_2$ emulsion (Table 1), which
347 agreed with the fact that this emulsion showed signs of coalescence after one day of storage.
348 Core/shell droplets stabilized with 2.86 wt% PGPR released only 16 % of sucrose during
349 digestion, which can be explained by the synergistic stabilizing effect of PGPR and NaCl.
350 There are many evidences that the presence of salt in the aqueous phase increases the stability
351 of PGPR-stabilized W/O/W and W/O emulsions (Márquez et al. 2010). The salt seems to
352 increase the elasticity of interfacial PGPR films and decrease its hydrophilic-lipophilic
353 balance (HLB) value by depleting the hydration shell around hydrophilic polyglycerol

354 moieties and thus, promoting hydrophobic interactions between PGPR chains. In real
355 applications, the salt content in the inner phase will be lower, which will likely lead to higher
356 release of sucrose compared to the amount of sucrose released in this work.

357 Core/shell droplets containing 1.4 wt% PGPR in the shell were less stable, releasing 53
358 % of sugar. Decreasing PGPR concentration decreases the interfacial tension at both oil-
359 water interfaces and lowers the middle phase viscosity, which leads to enhanced coalescence
360 of both internal and external droplets. Core/shell droplets stabilized with 0.7 wt% PGPR
361 released 49 % of sucrose during digestion, but the total amount of sugar released during
362 emulsification and oral processing was higher than with 1.4 wt% PGPR in the middle phase.

363 Micrographs of emulsion samples before and after digestion are shown in Figure 5.
364 Core-shell droplets stabilized with 2.86 wt% PGPR were monodispersed prior to *in-vitro*
365 digestion (Fig. 5a). After digestion, three different droplet morphologies can be distinguished:
366 the original droplets with core-shell morphology that survived the treatment, small oil
367 droplets with a diameter of 110 μm formed due to bursting of oil shells and large oil droplets
368 formed by coalescence of original oil droplets due to mechanical action and enzymatic
369 reaction during simulated oral processing (Fig 5. b-c). In the $(W_1/O)/W_2$ emulsion sample
370 (Fig. 5d), phase inversion was detected. The $(W_1/O)/W_2$ emulsion was inverted into
371 $W_2/(W_1/O)$ with large water droplets, with a diameter larger than 400 μm , dispersed in the
372 W_1/O emulsion. In the case of the $W_1/O/W_2$ emulsion with 1.4 wt% PGPR (Fig. 5 e-f), a
373 higher degree of coalescence was detected than when 2.86 wt% PGPR was used.

374

375 Fig. 5

376 **Conclusion**

377 Monodispersed $W_1/O/W_2$ emulsions consisting of starch-coated oil shells and aqueous
378 cores loaded with concentrated sucrose solution were successfully generated in a microfluidic
379 device. The droplet generation was stable for over 5 h and the size and morphology of the
380 droplets were controlled by varying fluid flow rates. A multiple emulsion consisting of
381 numerous tiny internal droplets stabilized with 2.86 wt% PGPR broke down rapidly during
382 *in-vitro* oral processing, releasing almost all sugar from the internal droplets, which is
383 beneficial to boost the sweetness perception, but the emulsion droplets coalesced after 1-day
384 storage. The emulsion instability during *in-vitro* digestion with α -amylase could be related
385 with the facility of this enzyme to be absorbed to the interface of this emulsion.

386 Core/shell droplets showed higher stability against coalescence than oil droplets with
387 numerous tiny internal droplets. However, only 16% of sucrose was released during *in-vitro*
388 digestion from the core-shell droplets containing 2.86 wt% PGPR in the shell. Such a low
389 sucrose release rate could also be attributed to the stabilizing effect of added NaCl. Reducing
390 the PGPR content in the oil phase improved sucrose release, but the encapsulation efficiency
391 of sucrose was also reduced. The use of molecularly dispersed OSA-modified starch instead
392 of gelatinized starch led to production of smaller droplets, due to lower interfacial tension in
393 the presence of interfacially active starch. On the other hand, gelatinized starch imparted a
394 long-term storage stability due to adherence of its large insoluble aggregates onto the external
395 droplet surface, which led to the formation of thicker interfacial layer.

396 The sugar contents applied in this study were selected to evaluate the feasibility of this
397 sweetness-enhancing technology. The sugar content in the actual food product will depend on
398 the proportion of internal emulsion in the final formulation and can be freely adjusted.

399

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401

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406

407

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Figure 1

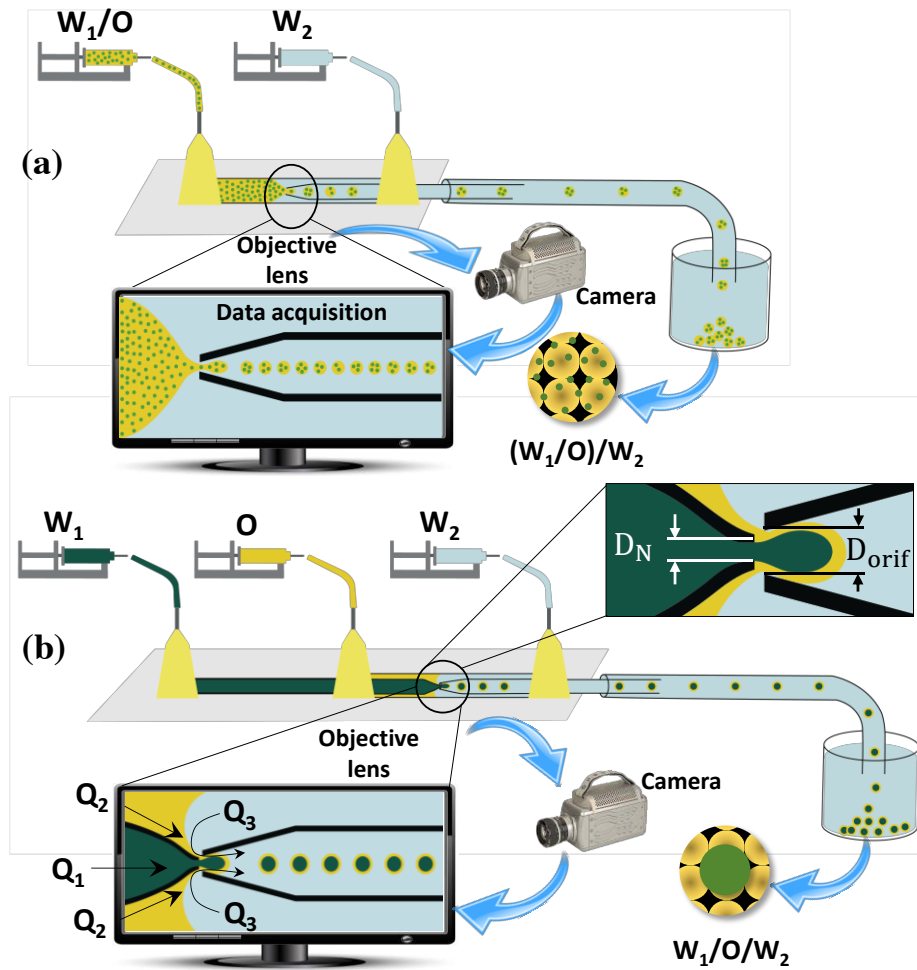


Figure 1. Schematic of the experimental setup consisting of glass capillary device, syringe pumps, inverted microscope and high-speed camera; (a) 2-phase device which requires two pumps for producing $(W_1/O)/W_2$ multiple emulsion consisting of numerous tiny inner droplets dispersed in oil drops; (b) 3-phase device which requires three pumps for producing $W_1/O_2/W$ multiple emulsion consisting of one large inner drop surrounded by oil shell.

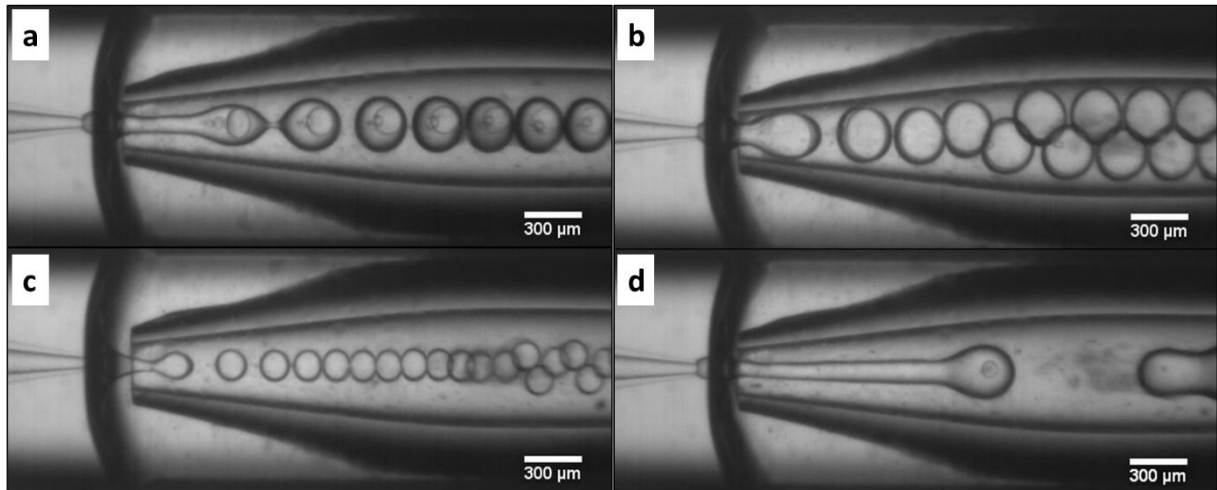


Figure 2. Formation of $W_1/O/W_2$ emulsion in a 3-phase device with the nozzle diameter $D_N = 50 \mu\text{m}$ and the orifice diameter $D_{orif} = 250 \mu\text{m}$ at different flow rates: (a) $Q_1 = 0.7 \text{ mL/h}$, $Q_2 = 2.3 \text{ mL/h}$, $Q_3 = 7 \text{ mL/h}$, $D_2 = 286 \mu\text{m}$, $D_1 = 157 \mu\text{m}$, $t_s = 64 \mu\text{m}$, $f = 9 \text{ Hz}$, $CV = 0.3 \%$; (b) $Q_1 = 2 \text{ mL/h}$, $Q_2 = 1.4 \text{ mL/h}$, $Q_3 = 3.5 \text{ mL/h}$, $D_2 = 299 \mu\text{m}$, $D_1 = 242 \mu\text{m}$, $t_s = 27 \mu\text{m}$, $f = 12 \text{ Hz}$, $CV = 0.2\%$; (c) $Q_1 = 2 \text{ mL/h}$, $Q_2 = 1.4 \text{ mL/h}$, $Q_3 = 15.5 \text{ mL/h}$, $D_2 = 160 \mu\text{m}$, $D_1 = 138 \mu\text{m}$, $t_s = 11 \mu\text{m}$, $f = 38 \text{ Hz}$, $CV = 0.2\%$; (d) $Q_1 = 0.1 \text{ mL/h}$, $Q_2 = 1.4 \text{ mL/h}$, $Q_3 = 7 \text{ mL/h}$. The inner phase was 1.5 wt % NaCl and 50 wt% sucrose in water, the middle phase was 2.86 wt% PGPR in sunflower oil and the outer phase was 25 wt% sucrose and 4 wt% gelatinised starch in water. The shell thickness t_s was calculated as $(D_1 - D_2)/2$, where D_1 and D_2 are the diameters of inner and outer drop, respectively.

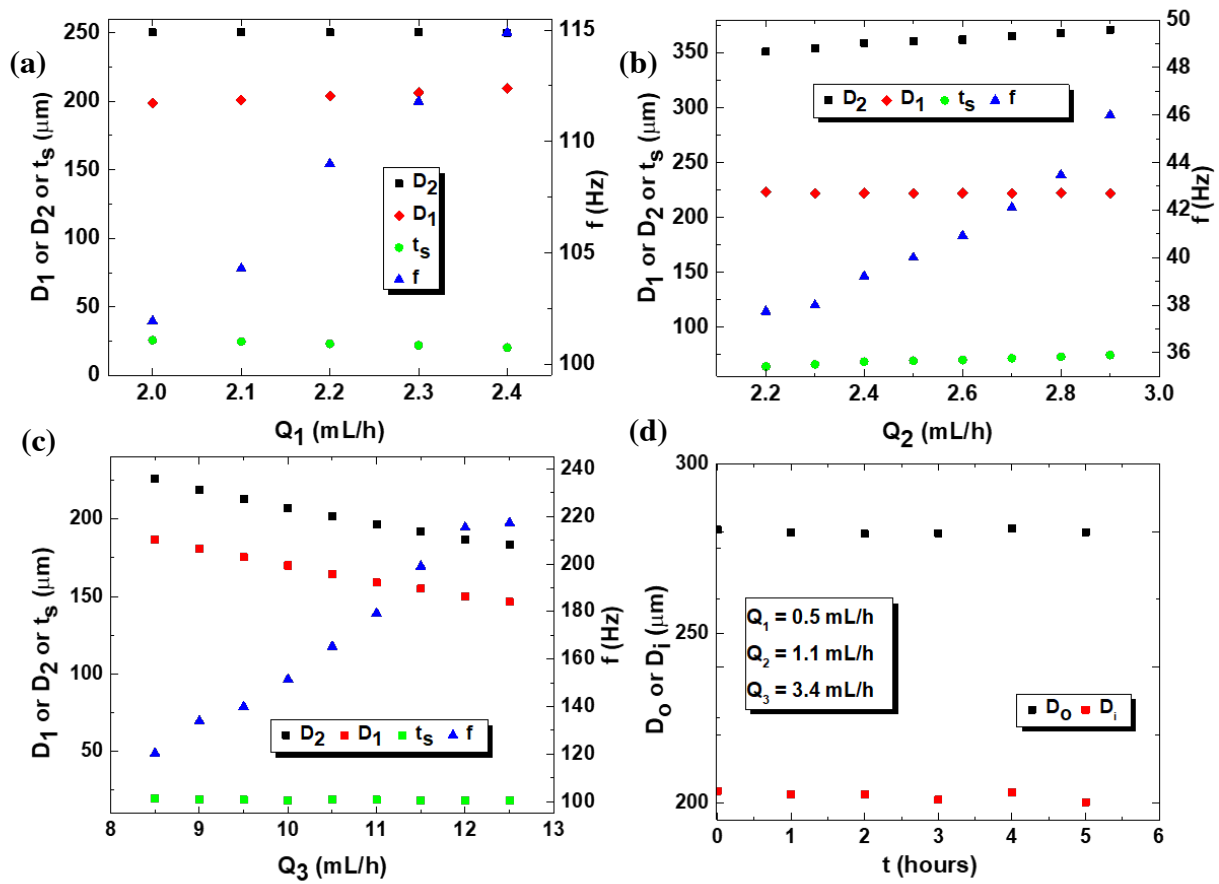


Figure 3. The effect of fluid flow rates on the diameters of inner and outer droplets, D_1 and D_2 , and generation frequency, f of $W_1/O/W_2$ emulsion formed in dripping regime at $D_N = 50$ μm and $D_{orif} = 250$ μm : (a) $Q_2 = 1.4$ mL/h and $Q_3 = 7$ mL/h; (b) $Q_1 = 1.2$ mL/h and $Q_3 = 5$ mL/h; (c) $Q_1 = 2$ mL/h and $Q_2 = 1.4$ mL/h. (d) The variations of droplet diameters during continuous droplet generation over 5 h. All droplet diameters are the average values from 20 measurements with $CV < 3\%$. The emulsion formulation is the same as in Figure 2.

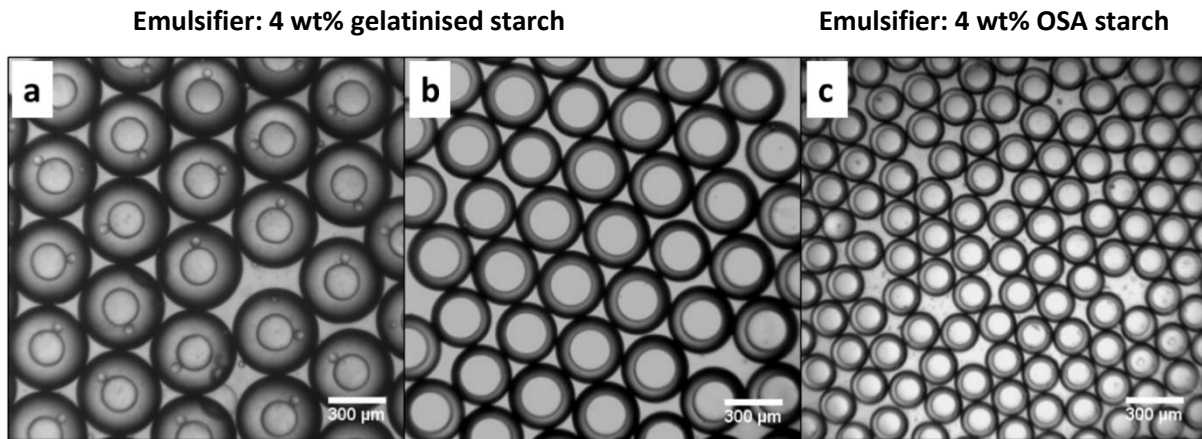


Figure 4. Microscopic images of W/O/W droplets with different sizes and shell thicknesses generated at: (a) $Q_1 = 0.2$ mL/h, $Q_2 = 2.5$ mL/h, $Q_3 = 5$ mL/h ($CV = 0.7\%$, $D_2 = 450$ μm , $D_1 = 190$ μm , $t_s = 131$ μm); (b) $Q_1 = 0.3$ mL/h, $Q_2 = 1$ mL/h, $Q_3 = 3.8$ mL/h ($CV = 0.9\%$, $D_2 = 343$ μm , $D_1 = 211$ μm , $t_s = 66$ μm); (c) $Q_1 = 0.5$ mL/h, $Q_2 = 1.5$ mL/h, $Q_3 = 3.4$ mL/h ($CV = 1.2\%$, $D_2 = 198$ μm , $D_1 = 116$ μm , $t_s = 41$ μm). The inner phase was 1.5 wt% NaCl and 50 wt% sucrose in water, the middle phase was 2.86 wt% PGPR in sunflower oil, and the outer phase was 25 wt% sucrose and 4 wt% starch in water.

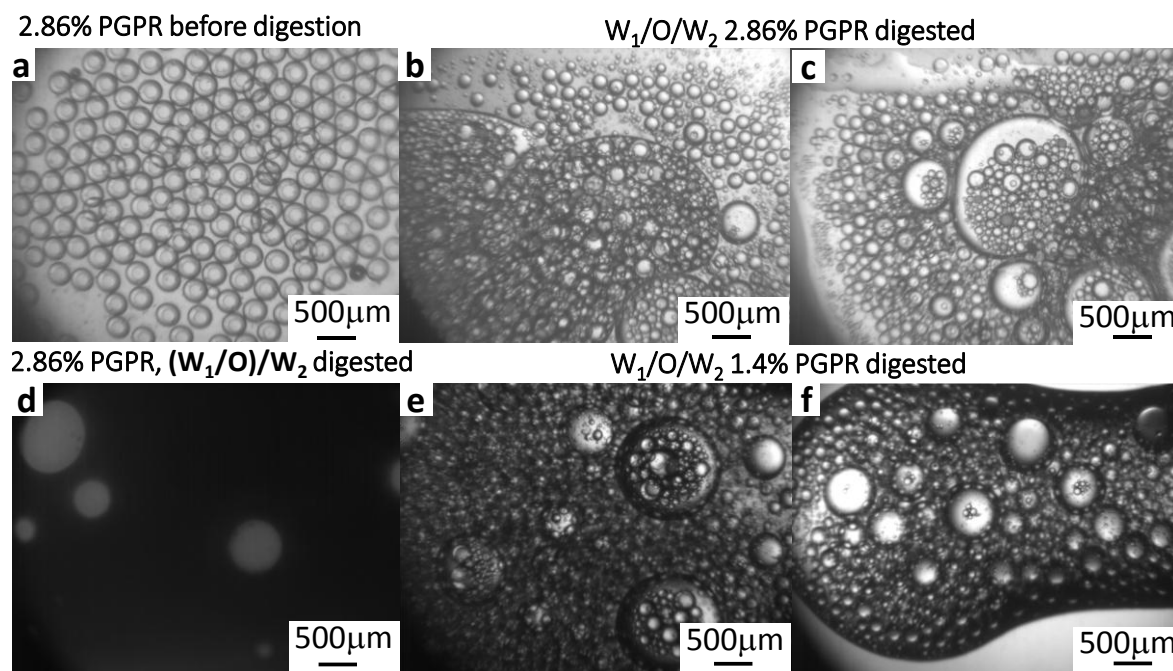


Figure 5. Micrographs of emulsion droplets before and after in-vitro oral digestion: (a) $W_1/O/W_2$ emulsion with 2.86 wt% PGPR in the middle phase before digestion; (b-c) $W_1/O/W_2$ emulsion with 2.86 wt% PGPR in the middle phase after digestion; (d) (W_1/O)/ W_2 emulsion with 2.86 wt% PGPR in the middle phase after digestion; (e-f): $W_1/O/W_2$ emulsion with 1.4 wt% PGPR in the middle phase after digestion.

Table 1. The efficiency of encapsulation, release of sucrose during *in-vitro* digestion with α -amylase, and stability against coalescence for different emulsion samples. The inner phase was 1.5 wt% NaCl and 50 wt% sucrose in water, the middle phase was PGPR in sunflower oil, and the outer phase was 25 wt% sucrose and 4% gelatinised waxy rice starch in water.

PGPR content in the middle phase and emulsion type	EE (%)	Sucrose release during digestion (%)	Emulsion stability		
			Day1	Day7	Day60
2.86 wt% PGPR, W ₁ /O/W ₂ [†]	100	16 ± 3	✓	✓	✓
2.86 wt% PGPR, (W ₁ /O)/W ₂ ^{††}	100	91 ± 10	✗	✗	✗
1.4 wt%, PGPR, W ₁ /O/W ₂ [*]	99	53 ± 4	✓	✓	✓
0.7 wt% PGPR, W ₁ /O/W ₂ ^{**}	70	49 ± 10	✗	✗	✗

[†] $D_i = 158 \mu\text{m}$, $D_o = 275 \mu\text{m}$, $t_s = 58 \mu\text{m}$; ^{††} $D_d = 291 \mu\text{m}$; ^{*} $D_i = 164 \mu\text{m}$, $D_o = 247 \mu\text{m}$, $t_s = 42 \mu\text{m}$; ^{**} $D_i = 187 \mu\text{m}$, $D_o = 305 \mu\text{m}$, $t_s = 59 \mu\text{m}$.

✓: denotes stable emulsion; ✗ denotes unstable emulsion.

All the results in the table present the average value of three replicates taken for two samples which were collected at different times during the emulsion formation process.

Method Details

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