

Effect of crystalline emulsifier composition on structural transformations of water-in-oil emulsions: Emulsification and quiescent conditions

Adam Macierzanka ^{**†§}, Halina Szeląg[†], Patrycja Szumała[†], Roman Pawłowicz[‡], Alan R. Mackie[§], Michael J. Ridout[§]

[†]Department of Fats and Detergents Technology, and [‡]Department of Chemistry, Technology and Biotechnology of Food, Chemical Faculty, Gdansk University of Technology, Narutowicza 11/12, 80-952 Gdansk, Poland;

[§]Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, United Kingdom.

Abstract

The objective of this study was to investigate the influence of acylglycerol (AG; mono-diglycerides) and acylpropyleneglycol (APG; fatty acid mono-diesters of propylene glycol) lipophilic emulsifiers, as well as their mixtures, on the structural transformations of emulsions, observed during emulsion formation and storage. The investigations aimed to characterize the phenomena that can be encountered during the manufacturing and shelf-life of emulsions prepared with these commonly used emulsifiers. It was found that in the presence of AG or AG-APG mixtures and up to 55–60 wt.% water content (depending on the emulsifiers proportions) continuous emulsification and cooling of the water-in-oil (W/O)

* Corresponding author. Tel.: +44 1603-255-242; fax: +44 1603-507-723.
E-mail addresses: adam_macierzanka@yahoo.co.uk,
Adam.Macierzanka@bbsrc.ac.uk (A. Macierzanka)

24 emulsion initially formed, led to the local phase inversion and finally formation of an oil-in-
25 water-in-oil (O/W/O) double emulsion with unique structure. The inversion was caused by the
26 temperature-dependent formation of the multilamellar liquid-crystalline structures of
27 monoglyceride in the aqueous phase of emulsion. The self-assembled structures encapsulated
28 oil droplets formed under the dynamic emulsification conditions and immobilized them in a
29 liquid-crystalline gel matrix. The resulting oil-in-water (O/W) emulsion gel was then
30 gradually dispersed in the oil phase, which was not involved in the formation of the internal
31 oil droplets. Finally, double O/W/O-type droplets were produced. Their structure can be
32 described as an O/W-emulsion lamellar-gel core surrounded by the interfacial layer of the
33 crystallized surface-active components of the emulsifiers. Such droplets coexisted in the
34 external oil phase with water droplets. When APG emulsifier was used as a sole stabilizer, no
35 phase inversion was observed. The W/O emulsion produced with this emulsifier underwent
36 crystallization- and shear-induced flocculation and subsequent deflocculation during the
37 emulsification. We also report on the time-dependent structural evolutions of the finally-
38 produced emulsions. The outcome of the multiple light scattering experiments and
39 microscopic observations was that emulsions based on the AG emulsifier or the AG-rich AG-
40 APG blends developed with time towards more tightly packed systems due to formation of a
41 fat-crystal network in the continuous oil phase. When the only emulsifier used was APG, the
42 structure of emulsion settled within three weeks, due to formation of crystalline bridges
43 between droplets.

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45 *Keywords:* Interfacial crystallization, double emulsion, phase inversion, liquid crystals,
46 rheology

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51 **1. Introduction**

52 Interfacial crystallization (at oil-water and air-water interfaces) and the ability to form
53 various liquid-crystalline phases with water are two very important features of
54 monoglycerides (monoacylglycerols, MAGs), which have an influence on their wide use in
55 many food applications, cosmetic products and pharmaceutical formulations [1-4]. The phase
56 behavior of saturated MAGs-water systems is relatively well understood [5-10]. Above the
57 Krafft temperature (the melting temperature of the hydrocarbon chains) the liquid-crystalline
58 lamellar phase is formed, which can transform into the cubic phase if the temperature is
59 further raised. Below the Krafft point, the lamellar phase transforms into the so-called α -gel
60 (highly-hydrated structure of the MAG bilayers with ordered hydrocarbon chains), which in
61 turn converts with time to the so-called coagel phase, a network of platelike crystals. Both gel
62 phases can be used in food products [11-13]. Since they differ in functional properties (e.g.,
63 hardness, foam stability), strategies for the preferential formation of one of these two phases
64 have been a subject of scientific investigations [13]. The kinetics of the α -gel→coagel
65 transition was studied mainly for binary MAG-water systems [13-19]. The transformation of
66 this type in the MAG-based ternary system, comprised water, oil and acylglycerol emulsifier,
67 was initially shown in our previous work [20,21]. More recently, the transition was studied by
68 Marangoni et al. for ternary gels composed of vegetable oil, water and monoglyceride/co-
69 surfactants [22,23]. The authors proposed that material as “a heart-friendly, low-saturate, trans
70 fatty acid-free” alternative to conventional spreadable fats and shortenings.

71 In many food and cosmetic applications, mono-diglycerides (a mixture of MAGs and
72 diacylglycerols, DAGs) are used. Both components are main products of either glycerolysis of
73 triacylglycerols (TAGs) or esterification of glycerol (G) with fatty acids (FAs), two main



74 industrial processes used for the production of acylglycerol emulsifiers [24]. The commercial
75 mono-diglycerides always contain some residues of TAGs, FAs and G. In some food products
76 (i.e., non-fat cake systems), MAGs are used in combination with another lipophilic crystalline
77 emulsifier, propylene glycol monostearate (PGMS), in order to make stable α -crystalline
78 blends of emulsifiers [1]. PGMS is an α -tending emulsifier [9,25]. It crystallizes in the α -
79 crystal form at about 39 °C [10]. PGMS is used in food industry as an effective emulsifier in
80 toppings, bakery shortenings and cake mixes [1]. In cosmetic formulations, it can be used as
81 an auxiliary emulsifier and viscosity modifier [26]. Like mono-diglycerides, fatty acid mono-
82 diesters of propylene glycol (PG) can be obtained by means of direct esterification of PG with
83 FAs or by transesterification of TAGs with PG. The latter process yields a complex mixture
84 of PG mono- and diesters and up to 10–15 % MAG, DAG and TAG as well as G and FAs,
85 and also some unreacted PG [9,24,27].

86 In oil-continuous emulsions, the emulsifying effectiveness of long-chain saturated
87 fatty acid monoesters of G and PG (e.g., monostearins, monopalmitins) is mainly due to their
88 ability to form mechanically strong crystalline films at the water-oil interface that prevent a
89 coalescence of dispersed water droplets [21,28,29]. The best protection of droplets (i.e., the
90 most effective coverage of the interface) can be obtained when the adsorbed fatty material
91 crystallizes directly at the interface [30], or in other words, when the crystallization follows
92 the adsorption step. Such a sequence can take place if the emulsification process is begun at
93 the temperature higher than the crystallization range of the surface-active substances and
94 followed by their solidification at quiescent conditions, or carried out under progressive
95 decrease of temperature throughout the entire crystallization range [21,29].

96 In this study, we found that the combination of in situ formation of liquid crystalline
97 phase and interfacial fat crystals determined the types of transitions in emulsion
98 microstructure during the emulsification. The primary goal of our work was to investigate the



99 effect of counterparts of the commercial acylglycerol and acylpropyleneglycol emulsifiers,
100 used separately and together in various proportions, on the character of structural transitions
101 of emulsions. We have reported the results of two sets of experiments, the first on the
102 temperature/shear-dependent transformations of emulsions during emulsification processes,
103 and the second on the post-crystallization changes in emulsion structure under quiescent
104 conditions.

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107 **2. Experimental**

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109 *2.1. Materials*

110 Acylglycerol (AG) and acylpropyleneglycol (APG) emulsifiers were synthesized by
111 means of uncatalyzed esterifications of respectively, glycerol (G; 99.5+% purity; Chempur
112 Company, Poland) or propylene glycol (PG; 99.5+% purity; POCH Company, Poland), with
113 stearic acid (SA; Sigma-Aldrich Chemie Gesellschaft GmbH & Co. KG, Germany). The SA
114 substrate consisted of 92.2 wt.% stearic (C18:0), 4.0 wt.% palmitic (C16:0), 1.8 wt.% shorter
115 and longer saturated and 2.0 wt.% unsaturated fatty acids. Total esterification procedures were
116 analogical to those described in the previous reports [29,31]. The initial molar ratios of G/SA
117 and GP/SA were 1:1 and 1.25:1, respectively. The compositions of the emulsifiers prepared
118 (determined by GC and HPLC) were as follows; (i) AG emulsifier: 43.0 wt.% glycerol
119 monostearate (GMS), 25.4 wt.% glycerol distearate (GDS), 6.1 wt.% glycerol tristearate
120 (GTS), 15.3 wt.% SA, and 10.2 wt.% G; (ii) APG emulsifier: 48.7 wt.% propylene glycol
121 monostearate (PGMS), 14.3 wt.% propylene glycol distearate (PGDS), 25.1 wt.% SA, and
122 11.9 wt.% PG. The hydrophile-lipophile balance (HLB) of the emulsifiers was determined
123 experimentally via the emulsion comparison method, as described in detail previously [21].



124 The HLB numbers obtained were 4.0 and 3.4 (± 0.1), respectively for AG and APG. In this
125 study, AG and APG emulsifiers were used separately or as mixtures at various proportions,
126 from 9:1 to 1:9 w/w. Note, the name “AG/APG emulsifier” used in the text corresponds to the
127 equivalent mixture of both components. The water used for the preparation of emulsions was
128 purified in a ROpureST/NANOpure system (Barnstead, USA). The oil phase was composed
129 of paraffin oil and paraffin wax, 9:1 w/w (both supplied by Sigma-Aldrich).

130

131 *2.2. Preparation of emulsions*

132 Emulsions were prepared by stirring in a glass, thermostated emulsor, using a
133 mechanical stirrer (Model RZR 2021; Heidolph, Germany) equipped with a steel agitator.
134 Water and the paraffin/emulsifier mixture (10 wt.% of the emulsifier, with respect to the total
135 amount of oil and water phases) were separately heated to 75 °C, and subsequently water was
136 gradually added (within about 10 s) to the mixture of oil and emulsifier under continuous
137 stirring (500 rpm). The water:oil weight ratios ranging from 20:80 to 80:20 were examined.
138 Emulsification was carried out in a decreasing temperature range of 75–25 °C. Selected stable
139 emulsions were kept at room temperature (23 ± 1 °C) for further experiments.

140

141 *2.3. Phase diagrams*

142 During the emulsification, the electrical conductivity and the temperature of the
143 studied systems were continuously measured to determine the type of the emulsion formed
144 (i.e., water-in-oil, W/O, or oil-in-water, O/W), the phase inversion temperature, and an
145 eventual separation of the dispersed water phase leading to an emulsion breakage. The
146 conductivity was examined using a conductivity cell [32]. Samples of the systems were taken
147 at various stages of emulsification (i.e., at different temperatures) and immediately
148 microscopically studied. Sudden, considerable changes in the macroscopic appearance of the

149 systems (e.g., homogeneity, phase separation) were also investigated by visual inspection.
150 Moreover, changes in the apparent viscosity of emulsion were monitored in situ over the
151 whole temperature range of the emulsification process as described below in detail. Based on
152 the data obtained, two types of phase diagrams were formulated. The first one shows the
153 dependence of emulsion type/structure on the temperature of emulsification and the water:oil
154 phase ratio. In the second-type diagram, the temperature- and shear-dependent structural
155 transformations of emulsions have been displayed as a function of the relative concentration
156 of AG emulsifier versus APG emulsifier (irrespective of the AG:APG ratio, the total
157 amount of the emulsifiers was equal to 10 wt.% of both oil and water phases). The diagram of
158 this type was prepared for constant water:oil proportion of 40:60 w/w. The temperatures of
159 the corresponding transitions in emulsion structure were determined from three preparations
160 and showed a maximum standard deviation of ± 0.5 °C.

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162 *2.4. In situ rheological analysis*

163 The emulsification processes were repeated directly in an AR2000 rheometer (TA
164 Instruments, UK), which gave an opportunity to measure in situ the apparent viscosity of the
165 emulsions as they were being formed. The rheometer was equipped with a vaned rotor/cup
166 arrangement (12 mm and 15 mm radii respectively, bottom gap = 5 mm) operated in constant
167 rotation mode. Prior to experiment, the temperature-controlled cup was filled with the
168 appropriate amounts of emulsifier and oil/wax (as specified above) and heated to 80 °C. To
169 ensure the components were completely melted and mixed, a 3-min pre-shear was applied.
170 The premix was left undisturbed for 2 min before a pre-heated water phase was introduced
171 into the system in the amount required to give a desired water:oil phase ratio. The
172 emulsification was carried out over a decreasing temperature range 75–25 °C (45 min) at a
173 shear rate of 500 s⁻¹. The high shear rate was chosen to mimic the conditions of emulsion



174 preparation where a high stirring rate was required to thoroughly mix the system and produce
175 the emulsion. The apparent viscosity was measured every 20 s and expressed as a function of
176 temperature. The temperatures of the significant changes in viscosity, related to the transitions
177 in emulsion structure, were determined from three measurements of the emulsion systems
178 produced under the same conditions. The maximum standard deviation did not exceed ± 0.5
179 $^{\circ}\text{C}$. Control analyses were also done for the emulsions without paraffin wax as well as for the
180 pure paraffin oil.

181

182 *2.5. Microscopy*

183 Morphology of emulsions was investigated by using a BX-60 microscope (Olympus,
184 Japan), equipped with APO oil-immersion objectives and a DP-50 digital camera (Olympus,
185 Japan). The microscope was operated in differential interference contrast (DIC) or cross-
186 polarized light modes. In some cases the hydrophilic Evans Blue (1 % w/v aqueous solution)
187 or the lipophilic Sudan II (1 % w/v paraffin oil solution) dyes were used to expose the water
188 or oil phase, respectively. Samples of emulsions were transferred to a microscope well
189 slide. A staining Evans Blue or Sudan II solution was added on the top of the sample and a
190 cover slip was carefully placed on top of the well. The staining solutions were used in the
191 minimum amounts required to achieve the desired contrast between the internal phase and the
192 stained continuous phase of an analyzed system.

193

194 *2.6. Calorimetric measurements*

195 Differential scanning calorimetry (DSC) was applied to determine the crystallization
196 profiles of AG, APG and mixed AG/APG emulsifiers, in their mixtures with paraffin wax
197 (emulsifier:wax weight ratio = 5:3). Appropriate amounts of emulsifiers and paraffin wax
198 were weighted into 20-mL glass tubes, heated to about 100 $^{\circ}\text{C}$, thoroughly stirred and cooled



199 to room temperature. Samples of 30 mg were weighted into 40- μ L aluminum pans,
200 hermetically sealed and stored 24 h at room temperature prior to measurements. Thermal
201 analyses were conducted on a Mettler TA 3000 calorimeter (Mettler Instrumente,
202 Switzerland) equipped with a TC 10 TA processor and a DSC 30 temperature cell. After
203 heating to 80 °C and keeping at this temperature for 5 min, samples were cooled to 0 °C at the
204 rate of 2 °C min⁻¹. An empty pan was used as a reference.

205

206 *2.7. Multiple light scattering*

207 The kinetics of emulsion structure being built under quiescent conditions (i.e., storage)
208 was determined at 23 \pm 0.5 °C by the multiple light scattering method, using a Turbiscane
209 LAb Expert analyzer (Formulacion, France). A freshly-prepared emulsion (ca. 25 mL) was
210 placed into a flat-bottomed cylindrical glass cell and stored at 23 \pm 1 °C. The sample was
211 scanned by using two synchronous optical sensors that detected the intensity of light
212 transmitted through and backscattered by the vertical sample (180° and 45° from the incident
213 laser light, respectively). The reading head acquired backscattering (BS) and transmission
214 data every 40 μ m while moving along the entire height of the cell (i.e., 55 mm). The light
215 source was an electro-luminescent diode ($\lambda_{\text{air}} = 880$ nm). As the emulsions studied were
216 opaque, only the backscattering measurements provided useful data.

217 The intensity of backscattered light depends on the photon transport length, λ^* , through
218 the dispersed system:

219

$$220 \text{BS} \approx [(1-B)/3] (h/\lambda^*)^{1/2}$$

221

222 where $B \approx 0.2$ is the boundary reflection factor in cylindrical geometry and h is the detector
223 height. The parameter λ^* corresponds to the penetration of photon into a dispersed system



224 (i.e., the distance above which a photon is scattered in the backward direction). λ^* depends on
225 the physical features of dispersed phase (mean particle diameter, d , and particle volume
226 fraction, ϕ):

227

$$228 \lambda^* = 2d / [3\phi(1-g) Q_s]$$

229

230 The optical parameters Q_s and g are given by the Mie theory [33].

231 Changes in BS values correspond to changes in the structure of an analyzed sample
232 [34,35]. For instance, a decrease in the total BS intensity usually indicates that the particle
233 size has increased due to phenomena such as flocculation/coalescence or Ostwald ripening. In
234 the case of particle migration, the increase in BS levels can be observed at the top or bottom
235 of sample, depending on the type of migration, i.e., creaming or sedimentation, respectively,
236 since the concentration of particles has increased in those regions. It is obvious that creaming
237 (or sedimentation) influences the simultaneous decrease of particle concentration at the
238 bottom (or in the uppermost region) of a vertical sample, and this is recorded by the analyzer
239 as a progressive decrease of BS intensity for that region. As long as BS readings are constant
240 for the entire height of a dispersion vertical sample, mean BS values are acceptable for
241 computation for particular scans recorded at different time points. In our investigations, the
242 monitoring of the microstructural behavior of emulsions was performed as a function of time.
243 The parameters ΔBS_{5-50} and λ^*_{5-50} were employed to characterize the kinetics of changes in
244 emulsion structure. ΔBS_{5-50} has been defined as a difference in mean BS value (recorded for
245 the emulsion sample in the region from 5 to 50 mm of its height; BS_{5-50}), between the first
246 scan and n scan. Whereas, λ^*_{5-50} corresponds to the mean value of the photon transport length
247 through the emulsion for the same region of the sample height. Results have been reported as



248 the structure change velocities, $d\Delta BS_{5-50}/dt$ or $d\lambda^*_{5-50}/dt$. Each sample was analyzed three
249 times, and the data are presented as an average.

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252 3. Results and discussion

253

254 3.1. Dynamic conditions: Emulsion formation

255 Fig. 1 shows the phase diagrams formulated for emulsions stabilized by (a) AG
256 emulsifier and (b) the equivalent amounts of AG and APG (AG/APG mixed emulsifier), as
257 well as the evolution of the apparent viscosity of the emulsions as observed during the
258 emulsification. The temperatures of the structural transitions of the emulsion being formed
259 were dependent on the presence of APG, however there were no significant discrepancies in
260 the types of transformations for the two cases. At the beginning of emulsification, W/O
261 emulsions were produced, which is an expected result for the use of strongly hydrophobic
262 emulsifiers. But after cooling to 59–58 °C (AG-based systems; Fig. 1a) or 56–54.5 °C
263 (AG/APG- based systems; Fig. 1b), a sudden significant decrease in viscosity was observed,
264 associated with rapid destabilization of the emulsions, i.e. separation of water and oil phases.
265 The transition is thought to be caused by the formation of the GMS-water or GMS/PGMS-
266 water lamellar gel phase. Indeed, gel structures were observed in the samples of the
267 destabilized systems (Fig. 2b). As the stirring was continued, oil droplets were formed and
268 entrapped within the gel matrix, resulting in the formation of the phase-inverted O/W
269 emulsion gel. This process and subsequent changes of emulsion microstructure are
270 schematically depicted in Fig. 2. Formation of the lamellar, MAG-water binary gels has been
271 a subject of many scientific reports, as mentioned in the Introduction. The lamellar liquid-
272 crystalline phase, produced with MAGs and/or different emulsifiers, was also found to be a

273 very effective stabilizer of O/W emulsions [9,22,23,36,37] due to its specific architecture
274 comprising the bimolecular layers of emulsifier separated by layers of water, which penetrates
275 into the planes of the polar headgroups of emulsifier molecules. The resulting lamellar gel is
276 capable of encapsulating oil droplets and, due to imparting steric hindrance between droplets,
277 prevents them from approaching each other and coalescing. With these facts as a basis, it is
278 not surprising that the formation of the gel phase, caused by a gradual cooling of the systems
279 studied here, produces the destabilization of W/O emulsions first and then the phase inversion
280 to O/W emulsions. The MAG-based gels can be produced from the lamellar mesophase when
281 the MAG-water systems are cooled below the Krafft temperature [9].

282 For low and medium water contents, (up to 55 wt.%) further cooling under continuous
283 emulsification led to another well-defined transformation, namely a sudden increase in the
284 viscosity of the system due to dispersion of the O/W emulsion gel in the external oil phase
285 (Fig. 1). This change corresponded well to the onset of crystallization of both AG and
286 AG/APG emulsifiers in their mixtures with paraffin wax (Fig. 3). It should be noted that the
287 presence of the APG component in the AG/APG emulsifier caused the onset of the
288 crystallization of this mixed emulsifier to be recorded at lower temperature (Fig. 3b) than
289 observed for AG emulsifier (Fig. 3a). Thus, the crystallization of acylglycerols was delayed
290 by acylpropyleneglycols. This in turn influenced the beginning of dispersion of the O/W
291 emulsion gel in the oil phase to be observed at lower temperatures (50.5–49.5 °C, Fig. 1b)
292 compared to the temperatures obtained for the use of AG emulsifier (56.5–55.5 °C, Fig. 1a).
293 When cooled separately from AG, the APG emulsifier began to crystallize at about 40 °C
294 (with the peak temperature at about 38.5 °C, Fig. 3c). All the exothermic thermograms shown
295 in Fig. 3 were recorded for the blends of the particular emulsifiers with paraffin wax to show
296 the crystallization profiles of all the components of emulsions that crystallize in the course of



297 the emulsification processes. The emulsifier:wax weight ratio applied, 5:3, corresponds to the
298 ratio present in the 40 wt.% water systems.

299 The dispersion of the O/W emulsion gel in the oil phase might be promoted by the
300 onset of the interfacial crystallization of the glycerol esters, which were not involved in the
301 formation of the lamellar gel. The created systems consisted of large-size agglomerates of the
302 O/W emulsion gel suspended in the oil phase (Fig. 2c). Since they did not form uniform
303 mixtures in terms of appearance (the clear continuous-phase oil was easily observed by a
304 naked eye), the systems have been called 'O/W + O' dispersions (Fig. 1, 2c). At the
305 temperature of 48–46 °C or 40–38 °C, depending on whether AG or AG/APG emulsifier was
306 used, another significant increase in the viscosity was observed (Fig. 1), which might be
307 connected to the crystallization of the other compounds of the emulsifiers (e.g., GDS, GTS,
308 PG esters). Continuous emulsification under progressive crystallization yielded the gradual
309 break-up of the O/W emulsion gel agglomerates and further gradual increase of the viscosity
310 (Fig. 1). Finally, smooth viscous emulsions were formed containing water droplets and
311 dispersed O/W emulsion gel, both covered by the interfacial layers of crystallized lipids (Fig.
312 2d). These complex systems have been designated as 'W/O + O/W/O' emulsions (Fig. 1, 2)
313 since the O/W emulsion gel dispersed in the external oil phase formed droplets of the oil-in-
314 water-in-oil (O/W/O) type. Existence of some nonadsorbed fat crystals, formed probably
315 mainly of paraffin wax, GTS, GDS and PGDS (the latter in the AG/APG-based systems), and
316 suspended in the continuous oil phase or attached to the crystalline shells of droplets, was also
317 observed (data not shown). To further quantify the investigations, the effect of the paraffin
318 wax on the viscosity profile has been studied in the 40 wt.% water systems. In the presence of
319 the wax the viscosity of the finally-produced 'W/O + O/W/O' emulsions was found to be
320 higher than without it (Fig. 1). Since it crystallizes in the continuous oil phase, the wax
321 appears to add to the viscosity of that phase as well as to the effective volume of the dispersed

322 fraction, both effects likely to be responsible for the observed enhancement of an overall final
323 viscosity of the emulsions.

324 Some representative O/W/O droplets from the 'W/O + O/W/O' systems were selected
325 for another microscopic characterization, which gave more insights into their internal
326 organization. The droplets found in the emulsion specimens were gently squeezed by slowly
327 pressing down the cover slip by the microscope lens. This way the crystallized film of
328 emulsifier was destroyed and the core of the droplets leaked out into the oil phase and was
329 easily observed. The interiors of the examined O/W/O droplets comprised small oil droplets
330 surrounded by and suspended in the liquid-crystalline gel matrix. The existence and location
331 of lamellar liquid crystals were revealed in cross-polarized light as a birefringent pattern of
332 the droplets (adsorbed lamellar phase) and the gel matrix between them (Fig. 4). The
333 birefringent pattern is a characteristic feature of lamellar liquid crystal-stabilized emulsions
334 [38]. From the above we can deduct that two types of mechanical stabilizations were present
335 in the structure of the O/W/O droplets. The internal oil droplets were stabilized by the
336 lamellar liquid crystals, forming the layers oriented at the surface of the oil droplets, and
337 immobilized by the gel matrix between them, whereas the external oil/gel matrix interface
338 was stabilized by the surface-active lipid crystals that were formed in situ at the interface.
339 Thus the structure of the O/W/O droplet was the O/W emulsion lamellar gel core
340 encapsulated by the interfacial layer of the solidified lipids.

341 Both, the W/O- and the O/W/O-type droplets were found to be formed in the final
342 emulsion ('W/O + O/W/O' system) if AG emulsifier was used alone or in a mixture with
343 APG emulsifiers. From Fig. 5, the most apparent result of the use of different proportions of
344 AG and APG emulsifiers is a change in the temperature ranges of the particular transitions in
345 emulsion structure. In general, the higher the content of the APG component the lower the
346 temperature of the relevant crystallization-controlled transformations, which might be due to



347 aforementioned shift of the crystallization of mixture of AG and APG emulsifiers towards
348 lower temperatures compared to the crystallization of AG alone. The presence of the AG
349 component was crucial for the formation of the lamellar gel and the subsequent phase
350 inversion, however only 10 wt.% AG content in an AG-APG mixture was required to do that
351 (Fig. 5). When used separately, APG influenced different transformations of emulsion being
352 produced (Fig. 6). For the water contents up to 50 wt.%, they were as follows (with
353 decreasing temperature): (i) the initial formation of W/O emulsion at the temperature range
354 above the level of the crystallization of paraffin wax and APG emulsifier, then (ii) the
355 progressive decrease in the fluidity of emulsion, (iii) the formation of huge flocs of water
356 droplets, due to simultaneous interfacial crystallization of the emulsifier and shear-induced
357 collisions of droplets, leading to the formation of crystalline links between droplets (this
358 phenomenon was observed as a substantial increase in the apparent viscosity at 42-40 °C;
359 being the more significant the more concentrated the emulsion was), and finally (iv) the
360 shear-induced disruption of the flocs caused by the ongoing emulsification at the temperature
361 below the range of the emulsifier crystallization, which yielded the formation of an ointment-
362 like, smooth W/O emulsion. The water droplets produced were covered by the crumpled
363 crystalline film of APG emulsifier (Fig. 7). Compared to the emulsification processes carried
364 out with AG and AG/APG emulsifiers (Fig. 1), the main difference in the above
365 emulsification pathway was the lack of the phase inversion step. It might be caused by
366 different behaviors of GMS and PGMS in the presence of water. According to Krog [39], in
367 contrast to monoglycerides, propylene glycol esters can not associate with water into
368 mesomorphic phases.

369 In all final W/O emulsions (stabilized by APG) and ‘W/O + O/W/O’ systems
370 (produced with AG or AG/APG), partially-coalesced (or partially-divided) droplets were
371 observed (Fig. 8). Such a structure of droplets can be produced in two different ways, as

372 proposed in Fig. 9 (note, for AG or AG/APG emulsifiers, the scheme covers only the post-
373 phase inversion part of the emulsification, i.e., the progressive formation of ‘W/O + O/W/O’
374 emulsions from ‘O/W + O’ systems). During the emulsification, large droplets are deformed,
375 elongated and subsequently broken into smaller ones due to the shear applied (Fig. 9A). Thus,
376 for the successful formation of smaller droplets proper viscoelasticity of the interfacial film of
377 emulsifier is required. Emulsification carried out from high to low temperature can yield the
378 most effective decrease in droplets size if emulsifier is in a liquid or semi-liquid state over
379 some initial temperature range, able to effectively reduce the interfacial tension and supplied
380 in a concentration high enough to cover the new interfacial area being formed during high
381 shearing (adsorption from the oil phase). The emulsifier should finally produce the strong
382 protective barrier against coalescence (end of emulsification at low temperature) to maintain
383 final droplet size (Fig. 9A a→d’). However, on cooling the system under continuous
384 emulsification through the entire range of emulsifier crystallization, various shapes of droplets
385 can be obtained depending of their shape at the endset of the interfacial crystallization,
386 namely spherical and irregular, non-spherical droplets (Fig. 9Ad’ and Ab’, respectively), as
387 well as partially-divided droplets (Fig. 9Ac’), all stabilized by the solidified interfacial layer
388 of emulsifier. Considering the morphology of the partially-divided droplets (Fig. 8), similar
389 structures can be produced as a result of the stirring-induced collision of droplets leading to
390 flocculation and subsequent coalescence (Fig. 9B), but in this case the resulting structures
391 should be called the partially-coalesced droplets due to a different history of their formation.
392 If the interfacial film of emulsifier remains, at a certain temperature, in a liquid or semi-liquid
393 state and is weak enough to rupture after the collision of droplets, the droplets will merge
394 (Fig. 9B a→c). But if in the next step a continuously crystallizing film is not able to regain a
395 spherical shape then two (or more) droplets will finally form a partially-coalesced cluster
396 stabilized by the interfacial membrane of solidified emulsifier (Fig. 9Bc’). On the other hand,



397 the flocculation of droplets can also occur at the temperature at which the interfacial film of
398 emulsifier is sufficiently solidified and strong to prevent a coalescence, and then the
399 crystallized flocs can be produced (Fig. 9Bb'). Such flocs can be disrupted into individual
400 droplets under shear applied during the emulsification (orthokinetic flocculation →
401 deflocculation), as shown above for the use of APG emulsifier (Fig. 6). The kinetics of these
402 dynamic phenomena depends on the temperature at which flocculation took place and, as a
403 consequence, on the physical state of emulsifier, as well as on the energy of collision of
404 droplets, the amount of the crystalline material involved in the formation of links between
405 droplets, and the time-scale for which the flocculated droplets have been exposed to shear.

406 The shape of droplets also depends on the temperature-dependent changes in the
407 viscosity of the dispersed phase. In general, the higher the viscosity of the dispersed phase the
408 longer it will take to deform and break up droplets of that fluid to form smaller droplets [40].
409 It can explain why in the 'W/O + O/W/O' emulsions studied, the O/W/O-type droplets were
410 found to be, in general, larger than the coexisting water droplets (Fig. 2d). Namely, the
411 viscosity of water is thought to be much lower than the viscosity of the internal phase of
412 O/W/O droplets, which was an O/W-emulsion gel stabilized by liquid crystalline phase of AG
413 or AG/APG emulsifiers' components. Thus, it could influence a relatively long time-scale that
414 was required to deform and divide such droplets, which along with the progressive interfacial
415 crystallization could yield the considerable number of deformed or even partially-divided
416 large O/W/O droplets. It is obvious that a decrease of temperature can also have an influence
417 on a considerable increase of viscosity of the continuous oil phase itself. As a consequence, it
418 can lead to less effective emulsification and/or enhanced stability of flocs to an orthokinetic
419 deflocculation. The dynamics of changes in the viscosity of continuous phase is expected to
420 depend on the composition of that phase and an emulsifier. In our particular case, the
421 viscosity might be governed by the non-surface active components of the studied mixtures



422 that crystallized in the continuous oil phase, imparting the significant increase of the viscosity
423 of the phase on cooling. In the studied systems such components are thought to be paraffin
424 wax (component of the continuous oil phase), and GTS and PGDS, present in AG and APG
425 emulsifiers, respectively. All these substances can form three-dimensional crystalline
426 networks in oil phases [29,41-45].

427 In the AG- and AG/APG-based systems containing 55–65 wt.% water, the lamellar
428 phase-induced destabilization and phase inversion yielded a flocculated O/W emulsion or an
429 O/W emulsion with some separated oil and water phases ('O/W + (O + W)'), respectively
430 (Fig. 1). Microscopic examination of these systems gave some insight into possible way of the
431 formation of O/W/O-type droplets for lower water contents. The separation of oil and water,
432 commenced at about 59–58 and 56–54.5 °C, in respectively AG- and AG/APG-stabilized
433 systems (Fig. 1), resulted in the formation of regions where water was in excess, which in turn
434 might result in closing of the bilayers into spherical aggregates. Such a tendency is a
435 characteristic feature of the lamellar phase in excess of water [46]. The aggregates contained
436 oil droplets bound by the lamellar gel (Fig. 10). Such structures have been thought to be the
437 cores of O/W/O droplets in the 'W/O + O/W/O' systems. Once the core was formed, it was
438 being encapsulated by the polar lipids that were not involved in the formation of the lamellar
439 gel and began to crystallize at the external oil-aqueous gel interface, forming solidified film.
440 The lipids are supposed to be GDS, SA and some amount of GMS, as well as PGMS in
441 AG/APG-stabilized emulsions. The O/W/O-type droplets were further divided into smaller
442 droplets due to the ongoing shearing, as described above for Fig. 9.

443 As the water content was increased over 65 wt.%, the product of the phase inversion in
444 both AG- and AG/APG-based systems was an O/W dispersion (Fig. 1). The inversion was
445 identified from a rapid increase of the electrical conductivity of the system, from 0-0.1 $\mu\text{S cm}^{-1}$
446 ¹ for the W/O emulsion before the inversion to ca. 10 $\mu\text{S cm}^{-1}$ as the transition to the O/W

447 dispersion took place. The phase inversion was accompanied by a significant decrease of the
448 apparent viscosity (Fig. 1). Once this drop was observed, it was followed by a temporary
449 increase due to formation of the gel phase, and finally the viscosity settled at lower values as
450 the O/W dispersion was formed. In the both systems, the dispersions contained predominantly
451 large (up to 400 μm) oil droplets covered by the crystallized emulsifier, and plenty of fine,
452 submicron-size droplets (data not shown). The lamellar gel phase was located mainly between
453 some fine droplets, and thus could not act as an effective thickening agent for the whole
454 system. Due to such an organization, the large particles formed a cream layer within a
455 relatively short time (up to a few hours) following the emulsification. Over 60 wt.% water
456 systems prepared with APG as a sole emulsifier underwent different transformations (Fig. 6).
457 At temperatures higher than 50 $^{\circ}\text{C}$, a coarse W/O emulsion was produced. It contained large
458 water droplets that coalesced under continuous agitation. The water phase released was
459 immediately redispersed and new droplets were generated. When the temperature dropped
460 below 50 $^{\circ}\text{C}$ a decrease in fluidity of the emulsion was observed, associated presumably with
461 the progressive crystallization of paraffin wax in the continuous oil phase. At about 40 $^{\circ}\text{C}$,
462 droplets rapidly flocculated due to the sharp crystallization of the emulsifier (Fig. 3) and
463 emulsion collapsed when emulsification was continued at lower temperatures. For these high
464 water/oil proportions, the crystallized interfacial membrane of emulsifier of many water
465 droplets was destroyed due to the shear applied and the destabilized system appeared,
466 designated in the phase diagram as 'W/O + W' (i.e., remained W/O emulsion + separated
467 water; Fig. 6). The release of water was signalled by a sudden increase of the electrical
468 conductivity, from 0-0.1 to ca. 9 $\mu\text{S cm}^{-1}$. The above transformations are similar to those
469 described recently for APG emulsifier modified with zinc fatty acid carboxylates [29].
470 However, as shown previously [47], propylene glycol mono-distearate with admixture of
471 sodium stearate (5-11 %) can yield kinetically stable viscoelastic O/W emulsions for more



472 than 50 wt.% water contents. This is likely because of the formation of the multilamellar gel
473 phase composed of PGMS with strongly hydrophilic anionic surfactant, sodium stearate, and
474 water. Such a phase can encapsulate oil droplets, imparting both the steric hindrance (an
475 onion-like lamellar shell) and the ionic barrier (repulsion of charged encapsulated oil droplets)
476 against coagulation.

477

478 3.2. *Quiescent conditions: Structure building*

479 After the emulsification, selected stable emulsions were further analyzed under
480 quiescent conditions. Fig. 11 shows a development of the structure of the oil-continuous
481 emulsions, as recorded by the MLS method during the storage at 23 ± 1 °C. In the systems
482 based on APG and AG/APG emulsifiers, a progressive decrease in the intensity of
483 backscattered light (BS) was observed during the first 1-3 weeks. Possibly, the decrease was
484 caused by a restoration of the crystalline bonds between droplets destroyed during the
485 emulsification. Under quiescent conditions, relatively rapid sintering between droplets
486 covered by lipid crystals might result in some spatial reorganization of emulsions. In the
487 APG-rich systems, the droplets formed a three-dimensional network stabilized by solid
488 crystalline bridges (Fig. 12). Hence, the emulsions were easier to penetrate by the incident
489 light of the MLS analyzer because the gaps filled with the external oil phase were formed
490 between the sintered droplets. Thus, the intensity of BS was lower than for the freshly-
491 prepared systems. The emulsions stabilized by APG emulsifier obtained their final structure
492 within about 3 weeks and then the settled levels of BS remained constant for at least 2 months
493 (Fig. 11a). In the AG/APG-stabilized emulsions, the initial drop of the intensity of BS,
494 attributed to the sintering between droplets, was followed by an increase after the first week
495 of storage (Fig. 11b). An increase was also observed for the emulsions prepared with AG
496 emulsifier (Fig. 11c), thus it might be connected to the presence of the glycerol esters. It is



497 likely that in these systems the sintering of droplets as well as the existing oil-continuous
498 crystals (i.e., the crystals formed in the oil phase at higher temperatures during the
499 emulsification, likely GTS, GDS and paraffin wax) was accompanied by further crystal
500 growth in the oil phase. In the AG-stabilized emulsions the increase was most pronounced for
501 the low dispersed-water contents (20, 30 wt.%, Fig. 11b). Possibly in these emulsions the
502 emulsifier was in excess and its considerable amount crystallized in the continuous oil phase
503 bringing about a packed structure of the emulsion system. More significant increase in BS
504 was observed for the AG/APG emulsifier (Fig. 11b). It may be assumed that in emulsions
505 stabilized by both AG and APG, sintering of droplets and continuous-oil crystals might be
506 created by acylpropyleneglycols (PGMS and PGDS), since they crystallized at lower
507 temperatures than acylglycerols and paraffin wax (Fig. 3), and are expected to be compatible
508 to both the oil-continuous and the interfacial crystals. The compatibility of adsorbed
509 monoolein and sintering triglycerides (palm kernel fat) was previously shown by Johansson
510 and Bergenståhl for the dispersion of β' palm stearin crystals in soybean oil [48]. According
511 to the presented rheological and sedimentation studies, the increased adsorption of the
512 triglycerides might lead to the enhanced bridging of the fat crystals.

513 Application of shear (emulsification) broke crystal structures formed at some earlier
514 stages of emulsification, as mentioned in the previous section, and then they started to re-
515 associate under quiescent conditions due to a mutual adhesion. In all probability, the AG- and
516 AG/APG-based emulsions crystallized partly under shear and partly at rest, so the re-built
517 crystals could also act as nuclei for further growth of acylglycerols crystals during the storage.
518 Finally in 'W/O + O/W/O' emulsions obtained with the AG emulsifier and the AG-rich AG-
519 APG mixtures, fan-shaped crystals were formed (Fig. 13). They were located in the external
520 oil phase, between water droplets. All the above post-emulsification processes, i.e., re-
521 association of existing crystals, their sintering and the sintering of the crystallized shells of



522 water droplets, and new crystals growth might occur simultaneously. Formation of the new
523 crystals in the continuous oil phase yielded a considerable increase in the effective volume
524 fraction of emulsions, which was recorded as a progressive enhance of BS values with time
525 (Fig. 11b, c). The influence of relative AG-APG proportions on the development of emulsion
526 structure has been shown in Fig. 14. The ratio determined the structure of the freshly-prepared
527 emulsions and the type of the structural changes observed during the storage. The parameter
528 λ_{5-50}^* (the mean photon transport length through emulsion) recorded for the freshly-prepared
529 emulsions was dependent mainly on the degree of dispersity of emulsions, increasing with the
530 increase of droplet size for the APG-rich systems. The formation of network of droplets,
531 observed during the first days of storage allowed photons of incident light to penetrate deeper
532 into the examined samples, so the parameter λ_{5-50}^* increased. When the AG content was more
533 than 10 wt.% in AG-APG mixture, the increase was followed by a step-by-step decrease,
534 which corresponded to the aforementioned post-emulsification formation of the aggregates of
535 crystals and, especially, the new crystals growth in the external oil phase. The emulsions were
536 kinetically stable for at least two months at room temperature. After this time, their structures
537 varied from a meshed network of coherent continuous-oil crystals and water droplets in the
538 AG-rich systems to huge droplets linked by crystal bridges in the APG-stabilized emulsions
539 (Fig. 12). Thus in the latter, the dominant post-crystallization process was the formation of
540 solid crystal bridges between crystalline membranes of droplets, whereas in the presence of
541 acylglycerols, the sintering of the continuous-oil crystals as well as the new crystals growth in
542 the oil phase could also take place. The first sign of destabilization, an oiling-out, was
543 observed after about 80 days in the 20 wt.% water emulsions. More concentrated emulsions
544 were stable against gravitational migration of droplets and oil phase during the time-scale of
545 storage (up to 6 months). Coalescence and separation of water phase were not observed at that
546 time.

547

548 4. Conclusions

549 The above investigations showed how a microstructure of emulsion can be modified
550 by both the relative proportions of two emulsifiers crystallizing during the emulsification
551 process and the water/oil ratio. Among the systems produced, the most complex, and thus
552 interesting, seem to be 'W/O + O/W/O' emulsions. Compared to the early Friberg's study on
553 the phase equilibrium between oil, water and lamellar liquid-crystalline phase of MAG in
554 liquid-crystal stabilized emulsions [36], as well as the ternary systems of aqueous phase, oil
555 and surface-active lipid crystals, reviewed recently by Larsson [46], the 'W/O + O/W/O'
556 emulsions reported in this paper are examples of more complex systems, consisting of oil,
557 water, and both liquid-crystalline lamellar phase and polar lipid crystals located at the water-
558 oil interface. The emulsions are systems where three different types of mechanical
559 stabilization have been found to occur. The internal oil particles in O/W/O-type droplets were
560 encapsulated by the lamellar liquid crystals, whereas the external water-oil interface was
561 stabilized by the solidified layer of the surface-active lipid crystals. Both, the liquid crystals
562 and the lipid crystals were formed in situ during the emulsification. Third stabilizing
563 mechanism derived from the post-emulsification processes observed in the continuous oil
564 phase. These comprised the sintering of continuous-oil crystals, the sintering between the
565 crystallized films covering water droplets, as well as the new crystals growth. They led to the
566 formation of the three-dimensional network of crystals and water droplets, which protected
567 the system from gravitational migration of the dispersed particles for a considerable time.

568 The 'W/O + O/W/O' emulsions are a good example of how complex colloidal
569 structures can be created by controlled but simple processing. The emulsions may have
570 possible applications as the oil-continuous formulations where a controlled release of water-
571 soluble ingredients is required. Such components could be introduced to the system with



572 water at the beginning of emulsification and then incorporated into the gel matrix of the
573 finally-formed 'W/O + O/W/O' emulsions.

574

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581

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746

747 **Figure captions**

748

749 Fig. 1.

750 The phase diagrams (left-hand side) and the apparent viscosity profiles (right-hand side) of
751 the water-oil systems prepared with (a) AG and (b) AG/APG emulsifiers. The black-colored
752 areas show the temperature ranges at which a sudden decrease of the viscosity and a phase
753 separation were observed during the emulsification. The dashed arrows correspond to the
754 temperature ranges of the gradual shear-induced transition of 'O/W + O' system to 'W/O +
755 O/W/O' emulsion (both systems coexisted at that stage).

756

757 Fig. 2.

758 Schematic representation (the left-hand side column) and microscopic visualization of the
759 lamellar gel-induced phase inversion of W/O emulsion, and the formation of the O/W
760 emulsion gel-loaded O/W/O double droplets, in the systems produced with AG (the middle
761 column) and AG/APG (the right-hand side column) emulsifiers, under continuous
762 emulsification at decreasing temperature and for water content ≤ 55 wt.%. The white arrows in
763 the micrographs of the panel (b) indicate the gel phase formed in the water, whereas the black
764 arrows in the micrographs (c2') and (c3') show the localization of the gel phase in the 'O/W +
765 O' systems produced at lower temperatures ('W' and 'O' correspond to water and oil,
766 respectively). Samples shown in micrographs (b2, b3) and (c2, c3) were stained with Evans
767 Blue and Sudan II, respectively.

768

769 Fig. 3.

770 DSC crystallization profiles of (a) AG, (b) AG/APG and (c) APG emulsifiers in their
771 mixtures with paraffin wax (emulsifier:wax weight ratio = 5:3). The arrows indicate the peak
772 crystallization temperature of paraffin wax.

773

774 Fig. 4.

775 The squeezing visualization of the internal structure of representative O/W/O droplets in the
776 'W/O + O/W/O' emulsion systems (W:O = 40:60 w/w) obtained with (a) AG and (b)
777 AG/APG emulsifiers; internal oil droplets covered by multilamellar liquid crystal layers and
778 incorporated into liquid-crystalline gel matrix, as observed using DIC (upper images) and
779 cross-polarized light (lower images) microscopies. Birefringent patterns around droplets
780 (lower images) show oriented liquid crystals. 'W' and 'O' correspond to water and external
781 oil phase, respectively. The scale bar = 10 μm .

782

783 Fig. 5.

784 Effect of the relative proportions of AG and APG emulsifiers and the temperature on the
785 structural transformations of emulsions during the emulsification process. The water:oil
786 weight ratio = 40:60. The black-colored area shows the temperature range at which a sudden,
787 temporary decrease of the viscosity and a phase separation were observed. The dashed arrows
788 correspond to the temperature range of the gradual shear-induced transition of 'O/W + O'
789 system to 'W/O + O/W/O' emulsion (both systems coexisted at that stage).

790

791 Fig. 6.

792 The phase diagrams and the apparent viscosity profiles of the water-oil systems prepared with
793 APG emulsifier. The gray-colored area represents the temperature range of the progressive
794 decrease in the fluidity of emulsion before the interfacial crystallization of the emulsifier and

795 the flocculation of droplets took place. The dashed arrows correspond to the temperature
796 range of the gradual shear-induced deflocculation of W/O emulsion.

797

798 Fig. 7.

799 Interfacial crystallization of the APG emulsifier in W/O emulsion (40:60 w/w; (a) the system
800 obtained after cooling to 25 °C). Image (b) shows the magnified DIC view of the surface of a
801 representative water droplet. The scale bars correspond to (a) 30 μm and (b) 10 μm .

802

803 Fig. 8.

804 DIC micrographs of the partially-coalesced/divided droplets formed in 40 wt.% water
805 systems. Samples were taken at the end of emulsification (25 °C). Emulsifier used: (a)
806 AG:APG = 70:30 w/w, (b) AG:APG = 20:80 w/w, (c, d) APG. The scale bars correspond to
807 15 μm .

808

809 Fig. 9.

810 Schematic representation of the influence of the continuous emulsification and the
811 progressive interfacial crystallization of emulsifier in W/O-type emulsions on the possible
812 ways of formation of the partially-divided and partially-coalesced water droplets (Ac' and
813 Bc', respectively); Stirring and shearing leading to an elongation and break-up of droplets
814 (A), and the collision of droplets resulting in flocculation and coalescence (B). For more
815 details see text.

816

817 Fig. 10.

818 Spherical aggregates of the gel phase with incorporated oil droplets observed in the 60 wt.%
819 water systems obtained with the AG emulsifier for the temperature range of 56–33 °C (see

820 Fig. 1a). The aggregates are suspended in the continuous water phase. Micrographs (a) and
821 (b) show variations in the size of the aggregates produced. Scale bars correspond to 10 μm .

822

823 Fig. 11.

824 The time-dependent evolution of ΔBS_{5-50} values for the W/O emulsions stabilized by (a) APG
825 emulsifier and the 'W/O + O/W/O' emulsions produced with (b) AG/APG and (c) AG
826 emulsifiers. Water:oil weight ratio: (\circ) 20:80, (\bullet) 30:70, (\square) 40:60, (\times) 50:50 and (\blacklozenge) 60:40
827 (graph c only). Emulsions were stored at 23 ± 1 $^{\circ}\text{C}$.

828

829 Fig. 12.

830 Effect of the relative proportions of AG and APG emulsifiers on the microstructure of
831 emulsions (W:O = 40:60 w/w). After the emulsification from 75 to 25 $^{\circ}\text{C}$, the emulsions were
832 stored for 60 days at 23 ± 1 $^{\circ}\text{C}$. The arrow indicates an example of the crystalline bridge
833 formed between droplets in the APG-stabilized emulsion. The scale bar corresponds to 30 μm .

834

835 Fig. 13.

836 An example of the fan-shaped crystal formed in the continuous oil phase of the 'W/O +
837 O/W/O' emulsions. The micrograph was taken for the 40 wt.% water system prepared with
838 AG:APG (70:30 w/w). 'W' indicates the surface of water droplet. The scale bar corresponds
839 to 5 μm .

840

841 Fig. 14.

842 Time-dependent evolution of the emulsion structure (W:O = 40:60 w/w) expressed as changes
843 in the mean photon transport length through emulsion (λ_{5-50}^*). Effect of the relative

844 proportions of AG and APG emulsifiers (w/w); (●) 100:0, (○) 70:30, (■) 50:50, (□) 30:70,
845 (▲) 20:80, (◇) 10:90, (◆) 0:100. Emulsions were stored at 23 ± 1 °C.