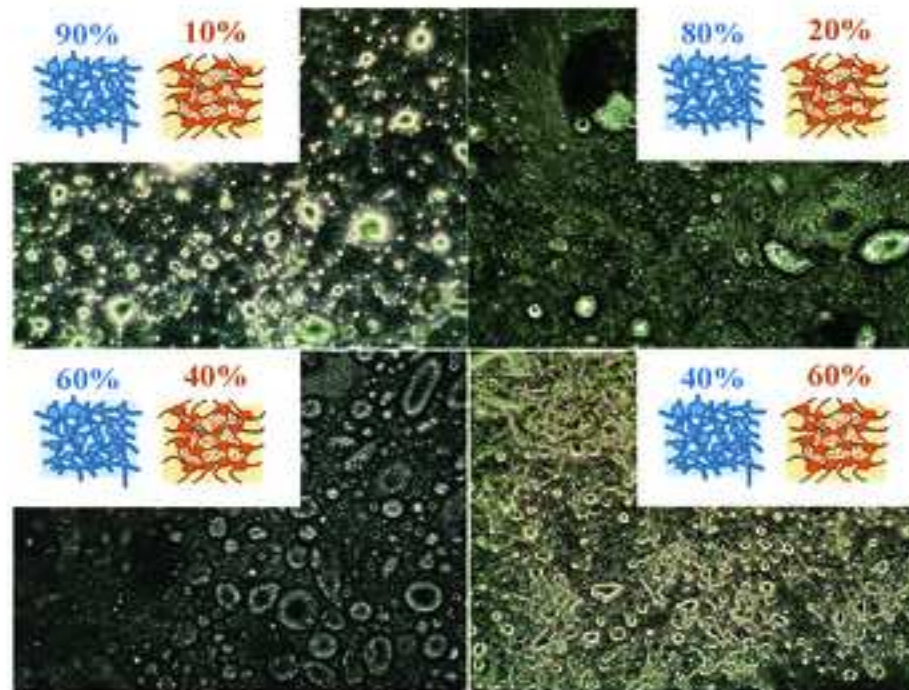


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HIGHLIGHTS

Cosmetic bigels were prepared with an olive oil organogel and a LM pectin hydrogel

Organogelators were a mixture of monoglycerides of fatty acids and fatty alcohols

An increase of organogel fraction gives more consistent and structured materials

Complex modulus is a function of organogel/hydrogel ratio and of their properties

An empirical model was proposed to relate bigel rheology to single phases properties

1 **A rheological and microstructural characterisation of**
2 **bigels for cosmetic and pharmaceutical uses**

3

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26 **ABSTRACT**

27 Bigels are biphasic systems formed by water-based hydrogels and oil-based organogels,
28 mainly studied, in the last few years, for pharmaceutical and cosmetic application
29 focused on the controlled delivery of both lipophilic and hydrophilic active agents. The
30 rheological properties of bigels depend on both the amount and the rheological
31 characteristics of single structured phases. Moreover, it can be expected that, at large
32 fractions of one of the starting gels, systems more complex than oil-in-water or water-
33 in-oil can be obtained, yielding bicontinuous or matrix-in-matrix arrangement. Model
34 bigels were investigated from a microstructural (i.e. microscopy and electrical
35 conductivity tests) and rheological point of view. The hydrogel was prepared by using a
36 low-methoxyl pectin whereas the organogel was prepared by using olive oil and, as
37 gelator, a mixture of glyceryl stearate and policosanol. Model bigels were obtained by
38 increasing the amount of organogel mixed with the hydrogel, and microstructural
39 characterisation evidenced an organogel-in-hydrogel behaviour for all investigated
40 samples, even though at the highest organogel content a more complex structure seems
41 to arise. A semi-empirical model, based on theoretical equations developed for
42 suspensions of elastic spheres in elastic media, was proposed to relate bigel rheological
43 properties to single phase properties and fractions.

44 **Keywords:** *bigel, organogel, hydrogel, cosmetics, rheology, olive oil*

45

46 1. INTRODUCTION

47 Bigels are biphasic systems, with enhanced mechanical and controlled delivery
48 properties, very interesting for food, but also very promising for cosmetics and
49 pharmaceutical uses. The main characteristic, with respect to other multiphase systems
50 (such as emulsions, emulgels, or filled gels), is that both phases (internal and external
51 one) are semisolid in nature [1-3]. In fact, they are mainly produced by mixing, at high
52 shear rates, an organogel and a hydrogel leading to the development of complex
53 matrices [2, 4-7].

54 In the case of topical use in cosmetic/pharmaceutical field, organogels are commonly
55 considered oily and not easy to be removed after application, even though they are
56 interesting for their ability to dissolve lipophilic agents. In contrast, hydrogels have high
57 market worth due to their patient compliance nature such as non-oily, cooling effect and
58 capability to be washed with water, but they can be used only as carriers of hydrophilic
59 components [8, 9].

60 Owing to their nature, bigels have the features of both gels, for example, the
61 improvement of hydration of the stratum corneum, the moisturising effect and good
62 spreadability, emollient properties and water washability upon application to the skin,
63 the ability to deliver both hydrophilic and lipophilic agents [2, 5, 6, 10]. The
64 contemporary use of two gel systems in bigels can give a synergetic effect such as
65 improvement of drug permeation due to the existence of both water phase and oil phase
66 [7]. Since bigels have the combined characteristics and advantages of organogels and
67 hydrogels, they may, possibly, be used as a topical drug delivery carrier on the skin in
68 pharmaceutical and cosmetic industries [5, 6, 11]. Moreover, owing to the structuration

69 of both phases of which they are composed, bigels do not display separation of the two
70 phases on storage at room-temperature for the duration of up to 6-12 months [7]. As
71 known, gels are stabilised by ensnarement of the mobile phases through a 3-D network,
72 and showing this microstructure in both phases, bigels are made of an extra-fine
73 dispersion of a phase into the other [3, 4, 7]. Bigel systems may have some
74 disadvantages, like they are not thermo-reversible as they may become destabilised at
75 high temperatures [7].

76 On the basis of the mutual disposition of organogel and hydrogel, bigels can be divided
77 into three categories:

- 78 i. Organogel in hydrogel system (O/W)
- 79 ii. Hydrogel in organogel system (W/O)
- 80 iii. Bi-continuous system

81 The first category of bigels includes the systems in which the oil phase is dispersed
82 within the aqueous phase and these systems are, probably, the most investigated in the
83 literature, where the hydrogel was prepared by using different kind of hydrogelators
84 such as starch, sodium alginate, sodium carboxy methyl cellulose [12], guar gum [7],
85 hydroxypropyl-methylcellulose [13], gelatin [1, 8], gelatin-agar mixture [1] or water
86 soluble synthetic polymers (e.g. polyvinyl alcohol and polyvinyl pyrrolidone) [2]. In the
87 same way different organogels were used as dispersed structured phase: Behera et al. [2,
88 12] used sunflower oil and Span 40 (Sorbitan Monopalmitate), the group of Singh [1, 7]
89 investigated a sorbitan monostearate-sesame oil based organogel. Ibrahim et al. [13]
90 proposed organogels based on soya-bean oil as a solvent and span 60, cetyl alcohol or
91 lecithin-pluronic as organogelators, mixed with other surfactants, and Sagiri et al. [8]

92 and Wakhett et al. [3] prepared organogels based on soya-bean oil as a solvent and
93 stearic acid as organogelator.

94 Bigels were characterised with different techniques aiming at investigating the material
95 microstructure and the mutual position of the structured phases. Rheological methods
96 were used by Behera et al. [2, 12], Singh et al. [7], Satapathy et al. [1]. Additional
97 techniques were also used such as fluorescence microscope [12], confocal laser
98 scanning microscope [7], compound optical microscopy [13], bright-field microscopy
99 [1, 3, 8], XRD, FTIR and DSC [8].

100 As far as hydrogel-in-organogel biphasic systems are concerned, a lower number of
101 works is available. Patel et al. [14] reported the characterisation of bigels made by
102 mixing fumed silica-sunflower oil organogel and polysaccharides (locust bean gum-
103 carrageenan) based hydrogel at different oil-water ratios. The bigel was studied with
104 confocal microscopy to understand the distribution of organic and aqueous phases, and
105 a discontinuous distribution of water phase in the oil phase was found.

106 Finally, Lupi et al. [15] investigated bigels produced by mixing a cosmetic formulation
107 (oil in water) for skin care with increasing fractions of monoglycerides of fatty
108 acids/olive oil organogels. NMR-self diffusion analysis, contrast phase microscopy and
109 electric conductivity confirmed that a complex matrix-in-matrix structure was present at
110 the highest fractions of added organogel.

111 Therefore, it seems evident that oil in water bigels at constant phase ratio are more
112 common, whereas less data are available on the effects of increasing the organogel
113 fraction and on potential transitions occurring in O/W systems with increasing
114 organogel content.

115 Starting from this point, the aim of the present work is the investigation of model O/W
116 bigels for potential cosmetic or pharmaceutical applications based on the controlled
117 delivery of hydrophilic/lipophilic active agents. These systems were prepared with
118 different phases ratios to understand their rheological properties and the relationship
119 between their mechanical characteristics with single phase properties.

120 Bigels were made with an organogel based on extra virgin olive oil and a mixture of
121 policosanol (a fatty alcohol mixture) and glyceryl monostearate (a monoglyceride of
122 fatty acids) as the organogelators. Different amounts of organogels were added to a low
123 methoxyl (LM) pectin hydrogel, and a rheological and microstructural analysis was
124 carried out (Small amplitude oscillation tests, Steady State Flow Curve Tests, Electric
125 conductivity tests, contrast phase microscopy). It was chosen to design new model
126 bigels starting from ingredients as much natural as possible (for example pectin as
127 hydrophilic gelling agent [16, 17], and olive oil [18, 19] as lipophilic solvent),
128 obviously chosen among those admitted for topical applications. In fact, the increasing
129 demand for clean label excipients [20] should be taken into account when designing
130 new formulations. In this framework, biopolymers currently used in the food field to
131 stabilise biphasic systems could be promising candidates in cosmetic/pharmaceutical
132 field too [20].

133

134 **2. MATERIALS AND METHODS**

135 Bigels were produced by preparing, separately, the hydrogel and the organogel, and
136 mixing them with a mechanical (rotor-stator) homogeniser at room temperature.

137 Samples with different organogel/hydrogel ratios were prepared and their properties
138 investigated.

139

140 **2.1 Organogel and Hydrogel materials**

141 The organogels were prepared with an extra virgin olive oil (EVO), kindly supplied by
142 Gabro (Italy), as the solvent, policosanol from rice bran wax (P) as the first
143 organogelator [21, 22], glyceryl stearate (GS), as the second organogelator/emulsifier
144 and tocopherol as an antioxidant. All the ingredients were supplied by A.C.E.F. (Italy).

145 Firstly, organogels were produced by adding tocopherol in the extra virgin olive oil to
146 prevent oxidation (the ratio tocopherol/olive oil was kept constant and equal to $6.15 \cdot 10^{-4}$
147 (w/w) according to a preliminary optimisation, data not shown), and then heating the oil
148 in a water bath thermostated by a plate heater (VELP Scientific, Italy) up to 85°C.
149 Afterwards, the gelators (i.e. P and GS in ratio 1:1) in the proper amount were added to
150 the hot oil, and the system was continuously stirred with a laboratory stirrer (RW 20,
151 IKA-Werke, Germany) at approximately 200 rpm. On complete melting of
152 organogelator, mixing was continued for 5 minutes. Finally, it was slowly cooled from
153 85 to 25°C in a thermostatic water bath (F25, Julabo, USA) and maintained at 25°C for
154 the time necessary to reach thermal equilibrium. Organogels were prepared by using
155 25% (w/w) (sample OM1) and 50% (w/w) (sample OM2) of organogelator mixture.

156 The hydrogel was prepared by using a LM pectin (Degree of Methoxylation 30.5%,
157 Galacturonic Acid content 89.9% and Molecular Weight 60.2 kDa) kindly supplied by
158 Silva Extracts S.r.l. (Italy), glycerine (Sigma Aldrich, Italy), citrate buffer solution with
159 pH equal to 4.2 ± 0.1 [23] (constituted of distilled water, citric acid, and tribasic sodium

160 citrate, Carlo Erba, Italy), sodium chloride and calcium chloride dihydrate (Carlo Erba,
161 Italy).

162 The buffer solution was prepared by mixing 0.85 g tribasic sodium citrate and 0.715 g
163 citric acid into 500 ml of distilled water (magnetic stirrer, Velp Scientific, Italy). Then,
164 2.9 g NaCl (corresponding to a final 0.1 M concentration) were added to the buffer (for
165 the electrical conductivity tests) and finally 0.0176 g calcium chloride dihydrate were
166 dissolved in the system to give gelation of LM pectin *via* calcium bridges between
167 carboxyl groups of the biopolymer, according to the well-known egg box model [23,
168 24].

169 The hydrogel was prepared by mixing pectin within buffer solution. In order to achieve
170 the best dissolution of pectin powder, preventing aggregate formation, pectin was
171 initially mixed with glycerine and then the buffer solution was slowly added into the
172 mixture at room temperature (25°C) , while stirring at 500 rpm for 2 minutes with an
173 Ultra Turrax homogeniser (UT T50, IKA-Werke, Germany). The composition of
174 hydrogel used for all the prepared bigels was fixed: in particular, it was made of 95%
175 (w/w) of aqueous solution, 1.25% (w/w) of pectin, the rest being glycerine.

176

177 **2.2 Bigels**

178 All bigels (batches of 200 g) were prepared by slowly incorporating the organogel into
179 the hydrogel at room temperature (approximately 25°C) while stirring at 1200 rpm for
180 8-12 minutes with an Ultra Turrax homogeniser (T50, IKA-Werke, Germany). Mixing
181 time was progressively increased, according to the increasing consistency of the
182 systems with higher amounts of organogel during the first part of the homogenisation

183 processes. Bigels were stored at 4°C for 24 hours, and afterward the tests were carried
184 out.

185 Table 1 reports the composition of all bigels. Samples BG1-BG4 were prepared with an
186 organogel (OM1) prepared with an equal mass fraction of gelators (i.e. 12.5% of each
187 gelator), the rest being olive oil, and the hydrogel previously described ('AP', aqueous
188 phase, within the text). Whereas, sample BG5 was produced with an oil phase
189 (organogel OM2) prepared with an amount of organogelators doubled with respect to
190 BG2, but maintaining the same organogel/hydrogel ratio used in BG2.

191 **2.3 Rheological characterisation**

192 **2.3.1 Organogel and Hydrogel**

193 The organogels and the hydrogel were investigated with a controlled stress rheometer
194 (DSR 500, Rheometric Scientific, USA) equipped with a parallel plate geometry
195 (diameter 40 mm, gap 1.0 ± 0.2 mm for organogels and 2.0 ± 0.2 mm for hydrogel) and a
196 Peltier system acting under the lower plate (± 0.1 °C). Frequency sweep tests, at 25°C, in
197 linear viscoelastic conditions, were performed by increasing frequency from 0.1 Hz up
198 to 10 Hz. In the case of organogels, with the aim of controlling the thermal history of
199 the sample, a temperature ramp test at constant frequency of 1 Hz was previously
200 carried out from 85°C down to 25°C in the linear viscoelastic regime and, after a rest
201 period of 100 s, the frequency sweep test was carried out. Preliminary stress sweep tests
202 were carried out at different temperatures from the preparation temperature, 85 °C,
203 down to 25°C, almost every 10 °C to examine the potential changes in linear
204 viscoelastic region, as a function of temperature.

205 **2.3.2 Bigels**

206 Bigels were examined with the stress controlled DSR 500 equipped with a parallel plate
207 geometry (diameter 40 mm, gap of 2.0 ± 0.2 mm). Preliminary stress sweep tests at
208 different temperatures were performed in order to evaluate the linear viscoelastic regime
209 and the potential changes with increasing temperature.

210 Dynamic temperature ramp tests (time cure tests) were, then, performed by heating the
211 sample from 10 °C up to 70 °C at 1°C/min, always ensuring the linear viscoelastic
212 conditions by modifying the applied stress with the temperature during the test
213 according to the results obtained in the preliminary stress sweep tests.

214 Frequency sweep tests at 25°C in linear conditions at frequencies ranging between 0.1
215 Hz and 10 Hz were carried out.

216 Finally bigel characterisation was completed performing flow curve tests at 25°C in
217 controlled stress conditions increasing the stress from 0.1 Pa up to 20 Pa. With the aim
218 of guaranteeing steady state conditions, a maximum measurement time of 900 s was
219 allowed for each point; to reduce the total test time, a “steady state sensing option” (RS
220 Orchestrator software, Rheometric Scientific) was adopted. Steady state was considered
221 to be reached when differences in transient measured viscosity values were lower than
222 2%, as already adopted for other complex materials [25].

223 Frequency sweep tests and flow curve tests were repeated three times on independently
224 prepared samples, whereas temperature ramp tests were repeated twice. All data are
225 shown in terms of mean values and standard deviations.

226 During all the rheological tests performed on water-based systems (i.e. hydrogels and
227 bigels), and especially in temperature ramp tests where high temperature of analysis

228 were used, water loss was prevented by covering the sample rim with a low viscosity
229 silicon oil (viscosity 30 mPa·s, Sigma Aldrich, Germany)

230 Samples were repeated at least twice, and data are given as the average values of the
231 results deduced from their characterisation. Data fitting was carried out with Table
232 Curve 2D Software (Jandel Scientific, USA).

233

234 **2.4 Microscopy Tests**

235 The microstructure of bigels in terms of mutual disposition of phases, and the particle-
236 size distribution (PSD), were investigated with an optical microscopy analysis. The
237 micrographs were obtained using a phase contrast microscopy (MX5300H, MEIJI,
238 Japan) equipped with phase contrast objective 40X. Among the investigated samples,
239 BG3 was analysed also with a polarised light microscopy filter. Micrographs were taken
240 both on undiluted samples (to better investigate the microstructure of bigels), and on
241 samples diluted with distilled water (1:10, volume fraction) and mildly stirred to break
242 aggregates. Dilution was necessary to make micrographs easier to be examined in order
243 to evaluate PSD. All samples were poured onto a glass slide inside a cover-imaging
244 chamber (Sigma Aldrich, Germany) on which a cover slide was put.

245 An image database software was used to measure the different sizes of particles (dhs
246 image database, Germany) by greyscale detection of micrographs taken on diluted
247 samples. The software measures the number based surface equivalent diameter, being
248 the diameter equal to diameter of a sphere with same surface area as particle. According
249 to a lognormal model used to fit experimental data, particles average dimension was

250 evaluated as the mean particle size \bar{d} , and the polydispersity by the standard deviation
251 of the distribution σ [26].

252

253 **2.5 Electrical conductivity analysis**

254 The disposition of gels within the bigels structure (O/W or W/O or Bi-continuous) was
255 also investigated by performing electrical conductivity analysis, as already done by
256 Lupi et al. in previous works on similar materials [15, 27]. In fact, all samples were
257 prepared with NaCl, which is able to conduct electricity in an aqueous phase, even if it
258 is structured as a gel. The electrical conductivity was measured at 100 Hz on parallel
259 copper plates (26x30 mm, gap 1 mm) with an LCR meter (Wayne Kerr, B905A, UK).

260

261 **3. RESULTS AND DISCUSSION**

262 **3.1 Rheological characterisation of organogels and hydrogel**

263 In order to investigate the effects of each phase on bigel properties, samples OM1 and
264 AP were studied. The results are displayed, in Fig. 1, in terms of complex modulus,
265 G^* ,(a) and phase angle, δ , (b) as a function of frequency.

266 It can be seen that both samples exhibit a typical gel behaviour; nevertheless, OM1
267 results to be much stronger than AP, which shows the typical characteristics of a weak
268 gel, being complex modulus a function of frequency [14, 28]. Moreover, AP possesses
269 G^* values much lower than those observed for organogels, whereas the phase angle
270 values are larger than those measured for the organogels at the same temperature.

271 Therefore, the aqueous phase adopted for bigel production is weaker and less structured
272 than the oil phase.

273

274 **3.2 Rheological characterisation of bigels**

275 The hydrogel above described was used as the structured aqueous phase of all bigels
276 whereas two different gelled phases (i.e. OM1 and OM2) were used: it is worth to
277 remember that OM1 was the gelled oil phase used for bigels BG1-BG4, obtained by
278 varying the organogel/hydrogel ratio according to data reported in Table 1. OM2 was
279 used in sample BG5 with the same oleogel/hydrogel ratio used for preparing BG2.

280 Complex modulus and phase angle at 1 Hz, from frequency sweep tests, of BG1-BG4
281 bigels are compared in Fig. 2 to data obtained for AP and OM1. The complex modulus
282 is presented in terms of G^*_r , the dimensionless ratio between complex modulus of each
283 sample at 1 Hz divided by the complex modulus of the hydrogel at 1 Hz.

284 It can be seen that the rheological characteristics of bigels are strongly influenced by the
285 organogel fraction and the increase in complex modulus with organogel fraction is not
286 linear; also the decrease of phase angle as a function of organogel fraction is non-linear.
287 Therefore, an increase of the organogel content, in these bigels, leads to an increase of
288 both the consistency of the material and the structuration degree, as evidenced by the
289 analysis of complex modulus and phase angle.

290 This increase in solid character of bigels, with organogel content, was already observed
291 in the literature [7] where stress relaxation tests, conducted with a mechanical tester,
292 were used to investigate the behaviour of samples based on guar gum hydrogels and

293 sorbitan monostearate organogels. It was found that increasing the organogel fraction
294 stiffer materials were produced. Moreover, if a simpler system is considered, i.e. a non-
295 diluted suspension, which could, with some effort, approach the rheological behaviour
296 of this kind of bigels, it is recognised that the increase of the particles volume fraction
297 corresponds to an increase of the elastic modulus of the system as well. Therefore, the
298 behaviour of oil-in-water bigels, from this point of view, could be expected [29].

299

300 **3.3 Thermal Stability**

301 A potential cosmetic application of investigated bigels imposes also the study of their
302 long-term stability. To assess the stability of biphasic systems, literature methods were
303 frequently based on accelerated procedures with the aim of reducing the time over
304 which the tests are performed [30] and, amongst the potential methods, ageing tests at
305 high temperature, usually 40 °C, for a constant period of time, are probably the most
306 used [31]. In recent times, rheology based methods were suggested as potential
307 alternative to traditional procedures, and it seems that dynamic temperature ramp tests
308 (time cure test) can be considered as effective methods to imitate the accelerated
309 destabilisation of cosmetic bigels [15]. Consequently, time cure tests were carried out
310 on bigels (BG1-BG4) in linear viscoelastic conditions and the results are shown in Fig.
311 3 (a and b). An initial region, where complex modulus is almost constant, can be
312 observed at low temperature. After a critical temperature, a relatively sharp decrease,
313 which can be considered as a beginning of destabilisation of the system, can be
314 observed. In a similar way, phase angle (Fig. 3b) is approximately constant at low
315 temperature and then increases, with increasing temperature, evidencing a continuous

316 reduction in solid-like component. At high temperature, after a peak, a slight reduction
317 is observed for all samples except for BG4 where the peak temperature corresponds to
318 the final test value.

319 The observed behaviour can be probably ascribed to the structural breakup of the bigels
320 because of the melting of the gelled phases (organogel and hydrogel) with increasing
321 temperature [15, 23], in fact both organogels and hydrogels melt at high temperature
322 (the exact values depend on gelator amount).

323 As approximate criterion, the melting point of the system can be considered as the
324 temperature correspondent to the phase angle value of 45° , indicating the crossover
325 between dynamic moduli (T_{cross}) and, therefore, the transition between a prevalent solid-
326 like behaviour and a prevalent liquid-like one.

327 All T_{cross} values are reported in Table 1; among samples produced with the same
328 organogelator/oil ratio, the most stable one is BG4, produced with the highest fraction
329 of organogel. On the other hand, for sample BG5 a crossover temperature was not found
330 in the investigated range, suggesting a greater stability of this system, probably because
331 of the increased content of organogelators used in the oil phase.

332 Figure 4 shows the time cure tests of BG2 and BG5 in terms of G^* and phase angle as a
333 function of temperature: it can be seen that the increase in the organogelator fraction
334 yields an increases of G^* and a reduction in phase angle suggesting that a more
335 consistent and more structured material is obtained. Moreover, phase angle of BG5 is
336 always lower than 45° evidencing the absence of a moduli crossover and, therefore, of a
337 melting point within the investigated range. This behaviour can be ascribed to the
338 increased organogelator amount: as already said, the melting point of these gels depends

339 on gelator amount and therefore it can be expected that it increases with increasing its
340 concentration.

341

342 **3.4 Flow curve tests**

343 All bigels showed a similar flow behaviour: at the lowest values of the applied stress, a
344 constant “linear creep region” (defined in this way by Barnes [32]), or zero-shear
345 viscosity η_0 is found (see Figure S1 in supplementary material). Increasing stress, it was
346 possible to measure the beginning of the narrow area between the linear creep region
347 and the non-linear one, just in samples produced with the lowest amount of organogel
348 (BG1-BG2). For the other samples slippage phenomena arose, limiting the measurable
349 stress-zone in the range 0.1-10 Pa. Zero-shear viscosity is a material property of the
350 system obtained in asymptotic kinematic conditions [25, 33]: it is independent of the
351 applied deformation history and, as a consequence, can be used as a parameter for the
352 characterisation of the investigated systems. It was calculated as the average viscosity
353 value within the linear creep region of the flow curves [25].

354 It can be seen (Tab.1) that zero-shear viscosity (and therefore material consistency)
355 increases with the organogelator amount in the oil phase (from BG1 to BG4 sample),
356 this confirms small amplitude oscillation tests, as already discussed. In a similar way
357 sample BG5, containing the same organogel amount of BG2 with a larger organogelator
358 fractions, evidences a value of η_0 larger than that of BG2. These results are in
359 agreement with data obtained from small amplitude oscillation tests confirming the
360 previous discussions on the effects of organogel and organogelator amounts on bigel
361 behaviour.

362

363 **3.5 Electric conductivity**

364 With the aim of verifying the mutual disposition of organogel and hydrogel, and to
365 further investigate the microstructure of the bigel, electric conductivity is a key issue. In
366 fact, the phenomenon of phase inversion, the sudden interchange of dispersed and
367 continuous phases of an emulsion, is experienced in the production, mixing, processing,
368 and handling of emulsions. Upon inversion of emulsions, a sudden change in the
369 electrical conductance of emulsion occurs [34]. The electrical conductivities (κ) of the
370 pure phases (organogel and hydrogel) and bigels were measured, and the ratio of the
371 value of κ for each bigel to the value of κ_0 , the electric conductivity of the pure
372 hydrogel, is shown in Tab.1.

373 Electric conductivity of pure organogel was found to be approximately zero ($5.88 \cdot 10^{-7}$
374 S/m), whereas for pure hydrogel κ_0 was 0.0147 S/m. Reported data clearly evidences a
375 relevant decrease of conductivity caused by the increased addition of the natural electric
376 insulant inside the hydrogel. When an oil phase is added to the system, no matter its
377 rheological characteristics, the electric conduction of the final material is, obviously,
378 reduced [15]. This consideration allows the investigated bigels to be considered as
379 organogel-in-hydrogel systems, in fact a phase inversion should lead to the electric
380 isolation of the system, being the hydrogel (conductor) confined in the organogelled
381 insulant matrix. On the other hand, no clear information on a potential more complex
382 behaviour (for example a bicontinuous structure) can be obtained from these data and,
383 therefore, a microscopy study was carried out.

384

385 **3.6 Microscopy tests and Particle-Size Distribution (PSD)**

386 The micrographs for all samples showed the presence of organogel particles suspended
387 within a continuous matrix of hydrogel, which confirms the formation of organogel-in-
388 hydrogel type of bigels (Fig. 5 for samples BG1-BG5).

389 Nevertheless, for the sample with highest organogel content (BG4), a more complex
390 structure, with some interconnections among organogel particles, seems to arise, even
391 though it seems that an organogel-in-hydrogel structure can be still observed. The
392 increasing complexity of bigels with the increase of oil phase is already highlighted by
393 the micrograph obtained with polarised light microscopy, which shows the
394 microstructure of sample BG3 evidencing an interconnection of particles, sometimes
395 stretched, and also the presence of air bubbles delimited by thicker boundaries.

396 The particle-size distribution parameters, mean diameter ' \bar{d} ' and standard deviation ' σ ',
397 for each bigels evaluated 24 hours after the preparation, are reported in Tab.1. As
398 already specified, PSD was evaluated starting from the diluted sample, and it is worth
399 noticing that pictures shown in Fig. 5 are referred to undiluted samples. Organogels
400 particles are smaller in samples containing lower proportions of the oil phase. There
401 was an increase in the particles size with an increase in the proportion of the organogel,
402 which is in accordance with the literature [4]. Standard deviation, which represents
403 polydispersity of particles, also showed similar trend: this experimental evidence is
404 probably attributable to the procedure adopted for bigel formation. When systems were
405 produced with a larger amount of organogel, mixing time was increased in order to
406 obtain a homogeneous material, nevertheless, owing to the increased consistency of the
407 starting material (because of the larger addition of organogel) more difficulties in

408 homogenisation and particle reduction were observed [23]. A comparison between the
409 PSD parameters for samples BG2 and BG5 indicates that, by increasing the total
410 amount of organogelator in the oil phase, particle size distribution does not change.
411 Moreover, the micrographs of both samples shown in Fig. 5 seem to confirm this trend.

412

413 **3.7 Rheological modelling of bigels**

414 The behaviour of bigels was only recently investigated in the literature, therefore, so far,
415 no specific model was proposed to relate rheological properties of these materials to the
416 characteristics of the single phases. On the other hand, bigels can be considered, from a
417 rheological point of view, as composite materials (or composite gels) having properties
418 depending on the fraction of dispersed particles and on the relation between the
419 viscoelastic properties of particles and of continuous phase [35-39]. Different models
420 were proposed in the literature to describe the rheological behaviour of similar systems
421 trying to take into account the most significant phenomena (shape and deformability of
422 fillers, particle distribution, etc.). The shear modulus of a concentrated solution of
423 elastic spheres in an elastic medium was calculated by van der Poel [40] and the
424 proposed equation was, afterwards, simplified to give an explicit equation where the
425 composite gel modulus depends on the ratio of shear moduli of dispersed particles and
426 matrix, M , and on volume fraction of filler, ϕ [39]. According to this simplified model,
427 for an incompressible matrix having a Poisson coefficient equal to 0.5, the
428 dimensionless complex modulus, i.e the ratio between the complex modulus of the
429 composite gel (G^*) and the complex modulus of the continuous phase (G_c^*), could be
430 described by the following equation [39]

431
$$G_r^* = \frac{G^*}{G_c^*} = \frac{1 + \frac{3}{2}H\phi}{1 - H\phi} \quad (1)$$

432 where

433
$$H = \frac{3(M-1)}{3M+3.5} \quad (2)$$

434
$$M = \frac{G_d^*}{G_c^*} \quad (3)$$

435 being G_d^* the complex modulus of the dispersed phase. A similar model was also
 436 proposed by Palierne [35, 38] for concentrated suspensions of spherical rigid particles,
 437 with H given by:

438
$$H = \frac{2(M-1)}{2M+3} \quad (4)$$

439 Eq. 1 was used to describe the complex modulus, at 1 Hz, of investigated bigels.
 440 Parameter H was computed according to both Eq. 2 and Eq. 4 without finding any
 441 numerical difference and volume fraction ϕ was computed as:

442
$$\phi = \frac{m_{og}}{m_{og} + m_{hyd} \cdot \frac{\rho_{og}}{\rho_{hyd}}} \quad (5)$$

443 m_{og} is the mass of organogel, ρ_{og} the density (980 kg/m³ at room temperature, data not
 444 shown), m_{hyd} the mass of the hydrogel and ρ_{hyd} the density (approximately 1000 kg/m³
 445 at room temperature).

446 The computed values of dimensionless modulus are compared to experimental ones in
 447 Fig. 2, it can be seen that the model underestimates the modulus and the difference

448 increases with filler content. This result is not so unexpected and it is also in agreement
 449 with some literature findings [39]. In fact, it seems that this model fails at high volume
 450 fractions [35, 39], moreover it was proposed for elastic undeformable spherical particles
 451 with unimodal distribution. The materials investigated in the present work are more
 452 complex than this “ideal” system, therefore, with the aim of describing in a simple way
 453 their behaviour, an empirical fitting model was proposed, modifying Eq. 1 and
 454 introducing the “deviation” from the ideal behaviour in two fitting parameters α and β :

$$455 \quad G_r^* = \frac{G^*}{G_c^*} = \frac{1 + \frac{3}{2} \alpha H \phi}{1 - \beta H \phi} = \frac{1 + a \cdot \phi}{1 - b \cdot \phi} \quad (6)$$

456 The application of Eq. 6 to the experimental results gave the following values of
 457 parameters a and b : $a=17.0 \pm 0.9$ (-), $b=1.627 \pm 0.001$ (-), and therefore $\alpha=11.3$ and $\beta \approx b$
 458 because $G_d^* \gg G_c^*$ and H is approximately equal to the unity. The model fits quite well
 459 the experimental data, as confirmed by the curve plotted in Fig. 2.

460 Organogel was considered as a dispersed phase and hydrogel as a continuous phase
 461 taking into account both microscopic and conductivity techniques. It is worth reminding
 462 that the above equation, due to empirical fitting, is only valid within the investigated
 463 range of experimental conditions, i.e. $0 \leq \phi \leq 0.601$. The model cannot be applied for the
 464 extreme point $\phi=1$. The model can be considered as an interesting empirical equation to
 465 predict the rheological behaviour of the considered system, within the investigated
 466 conditions, without performing other experimental investigations (that, in turn, would
 467 be necessary only if the raw materials would be changed). Obviously a deeper
 468 investigation of the relation between the model and the rheological properties of the
 469 pure gels could be useful to take into account better the effects of changes in single

470 phases, as a function of the amount of gelator, and the complex structure of bigels: in
471 this way, maybe, the current fitting parameters could be replaced by coefficients related
472 to the bigel morphology and structure.

473

474 **4. CONCLUSIONS**

475 Bigels, i.e. two phase systems where both phase are structured, were produced with an
476 organogel, containing glyceryl stearate (GS) and/or policosanol (P), and a low-methoxyl
477 (LM) pectin-based hydrogel by changing the ratio between the phases and increasing
478 the organogel (or the organogelator) content.

479 A preliminary rheological study of single phases was carried out to characterise the
480 bigel components. Organogels based on mixture of GS and P evidenced a behaviour
481 intermediate between those of “pure” organogels but much closer to that of P
482 organogels, in terms of both moduli, suggesting that policosanol is more effective in
483 organogelation than GS, whereas a typical weak gel behaviour was found to
484 characterise the hydrogel rheology. Rheological methods, microscopic studies and
485 conductivity measurements were performed to analyse the characteristics of the bigels
486 under investigation. Time cure tests showed that bigels containing higher fractions of
487 organogel or higher fraction of organogelators in the oil phase, were thermally more
488 stable owing to characteristics of the organogel that is more thermally stable with
489 respect to the adopted pectin gel.

490 Microscopy studies and electrical conductivity determination confirmed the
491 development of organogel-in-hydrogel morphology for the bigels, even if a more

492 complex structure seems arising for the highest fractions of organogels added to the
493 hydrogel.

494 Furthermore, the relationship between bigel rheological properties and single phases
495 characteristics was investigated. So far, in the literature, mainly emulsion gels or filled
496 gels were studied testing the validity of theoretical models often based on diluted
497 dispersion of single non deformable spheres or proposing empirical models. On the
498 contrary, in the present work, both phases are characterised by a viscoelastic behaviour
499 which affects the final bigel rheology. The experimental oscillatory data were,
500 preliminary, fitted by theoretical models which underestimate the experimental
501 behaviour, probably owing to deviation of the present system from the discussed
502 simplifying hypotheses. Then, a semi empirical model was proposed by modifying the
503 theoretical ones and introducing the deviation from “ideal” behaviour in two fitting
504 parameters.

505 The empirical model showed a quite good agreement with experimental data and
506 evidenced a clear dependence of bigels characteristics on the organogel fraction.
507 According to the obtained results, it seems that the investigated systems are promising
508 for cosmetic or pharmaceutical applications, where the topical delivery of active agents
509 could be controlled by the structuration of phases. In fact, even if it seems that there is
510 still a limited knowledge in the rheological and microstructural investigation of bigels in
511 general, being the number of papers discussing this topic already small, it is worthy
512 noticing that the majority of contributes available in the literature about biphasic
513 structured matrices is focused on their use for controlling the topical delivery of active
514 agents. In the light of this, a future analysis of the release of drugs or active agents
515 throughout the matrix in relation with its rheological properties, and the diffusional

516 phenomena involved in this process has to be carried out: from this point of view, this
517 work represent a first step in understanding bigels potentiality. In addition, a more
518 detailed rheological model could be useful to better describe the relationship between
519 macroscopic properties and single phases behaviour, and consequently, a further
520 investigation about the rheological behaviour of gels with different structure levels
521 could be necessary to improve the current knowledge on this topic. Finally, organogel-
522 in-hydrogel systems are currently much more investigated with respect to inversed
523 systems, and, as a consequence, a future analysis could involve also hydrogel-in-
524 organogel bigels for different applications and for the delivery of both lipophilic and
525 hydrophilic active agents, or also a mixture of them.

526

527 **5. ACKNOWLEDGMENTS**

528 The authors are grateful to Dr. Maria Converso for the execution of flow curve tests.

529

530

531

532 **TABLES CAPTIONS**

533

534 Table 1 Composition and main characteristics of bigels. T_{cross} is the temperature
535 at which a crossover between dynamic moduli occurs in a time cure test.
536 \bar{d} and σ are PSD parameters. 'n.d.' means that the parameter was not
537 determined for the specific sample

538

539 **FIGURES CAPTIONS**

540 Fig. 1 Frequency sweep tests at 25°C for samples OM1 and AP. Complex modulus, G^*
541 (a) and phase angle, δ (b) are shown.

542 Fig. 2 Complex modulus, G^* , and phase angle, δ , at 1 Hz for bigels as a function of
543 organogel fraction (pure samples are AP and OM1 respectively).

544 Fig. 3 Time cure test in linear conditions for bigels in term of complex modulus (a) and
545 phase angle (b) versus temperature. Dotted lines are models, symbols are experimental
546 values.

547 Fig. 4 Comparison between time cure tests for samples BG2 and BG5

548 Fig. 5 Contrast phase microphotographs for samples **BG1-BG5**: sample BG3 is shown
549 with both contrast phase microscopy (BG3 – cfm) and **polarised** phase microscopy
550 (BG3 – plm). Reference bar corresponds to 5 μm

551

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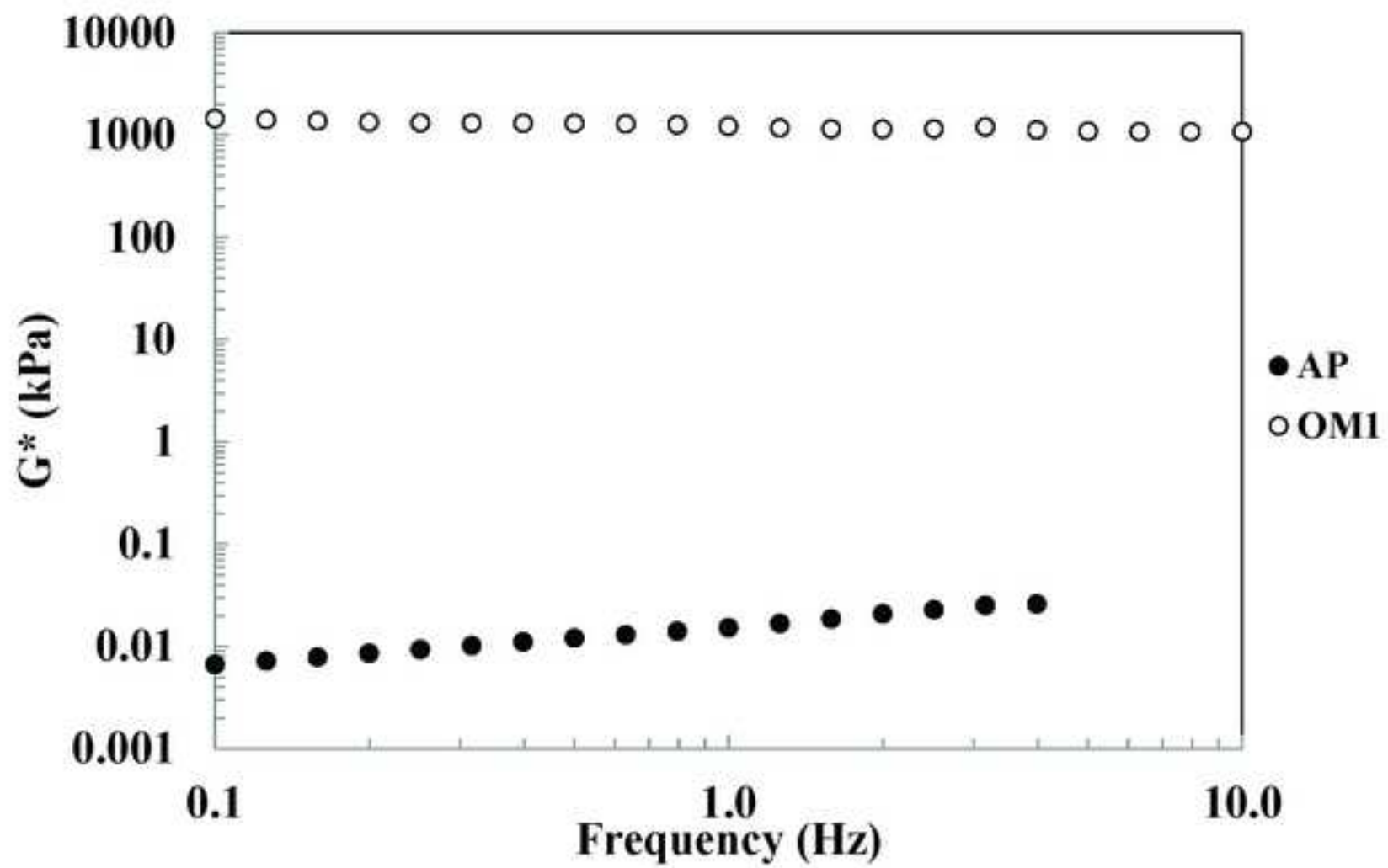


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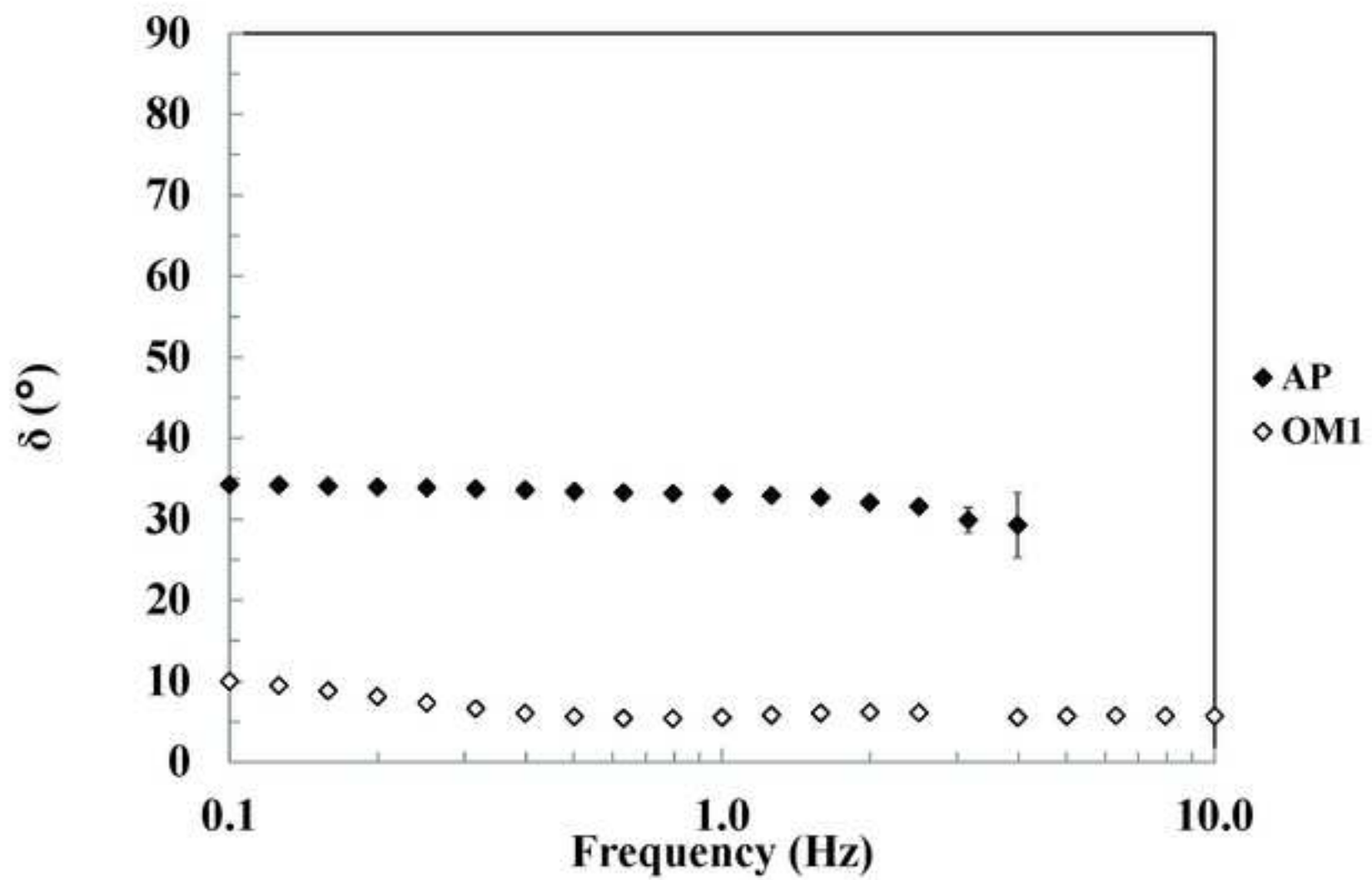


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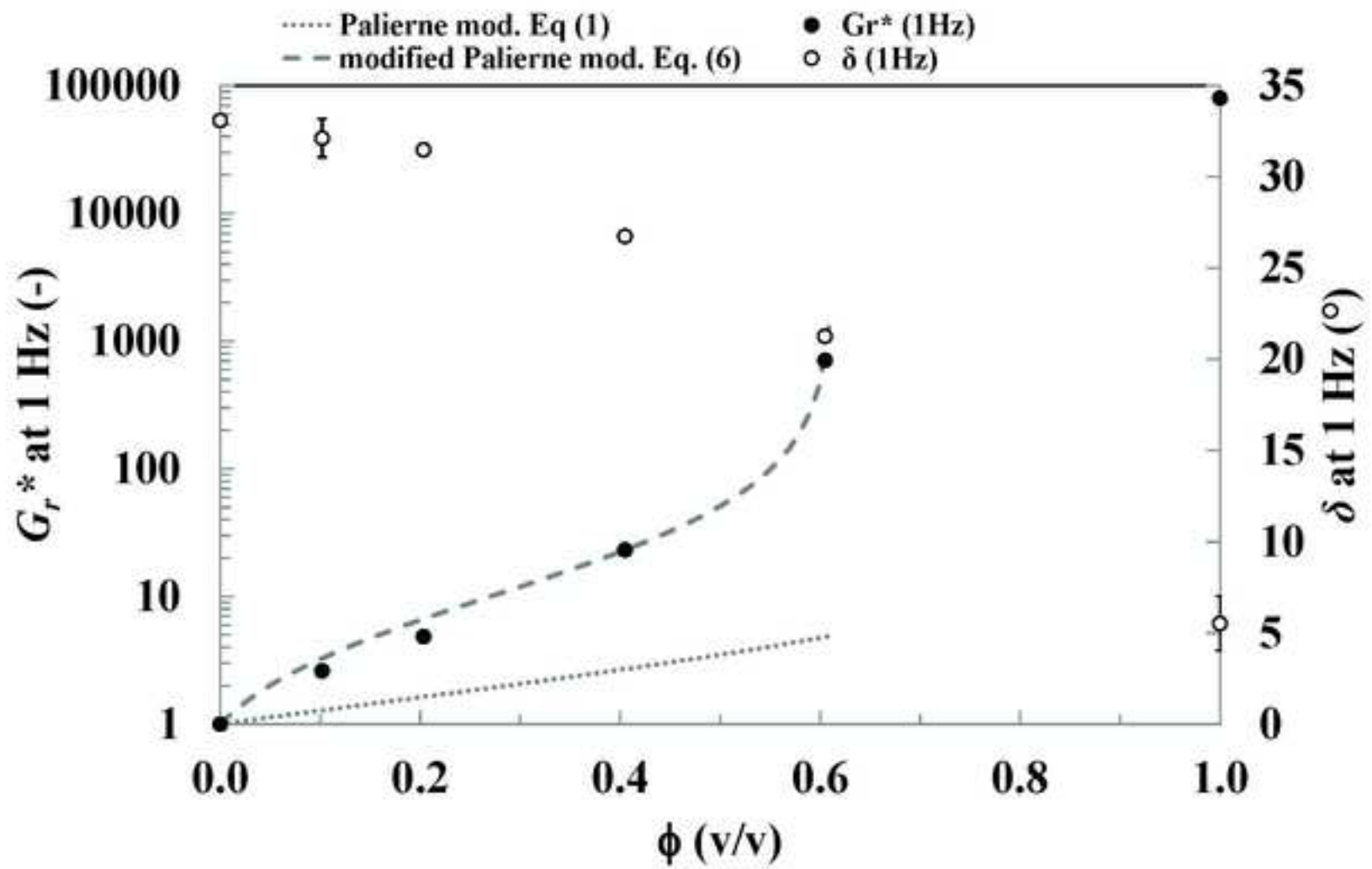


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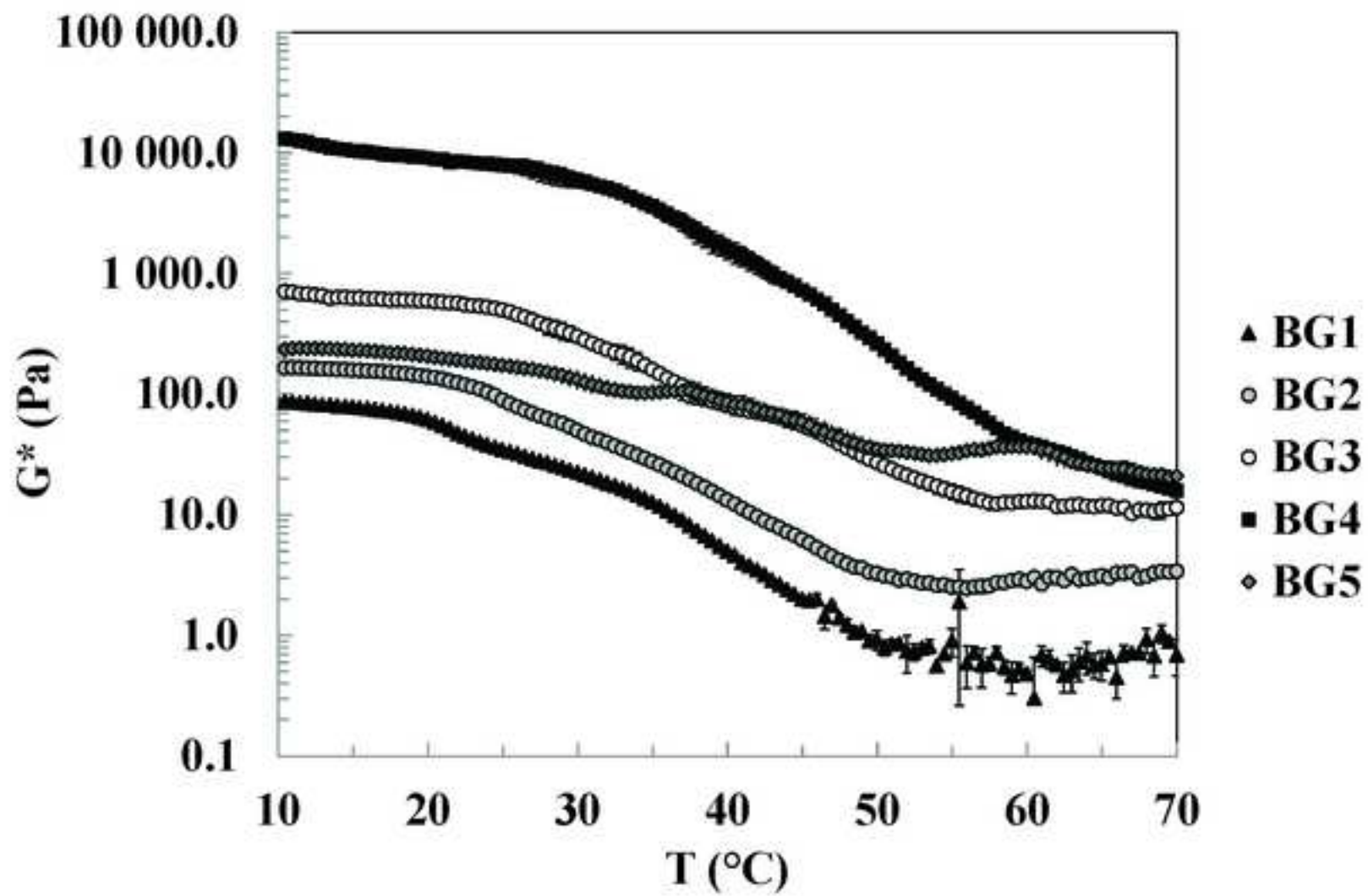


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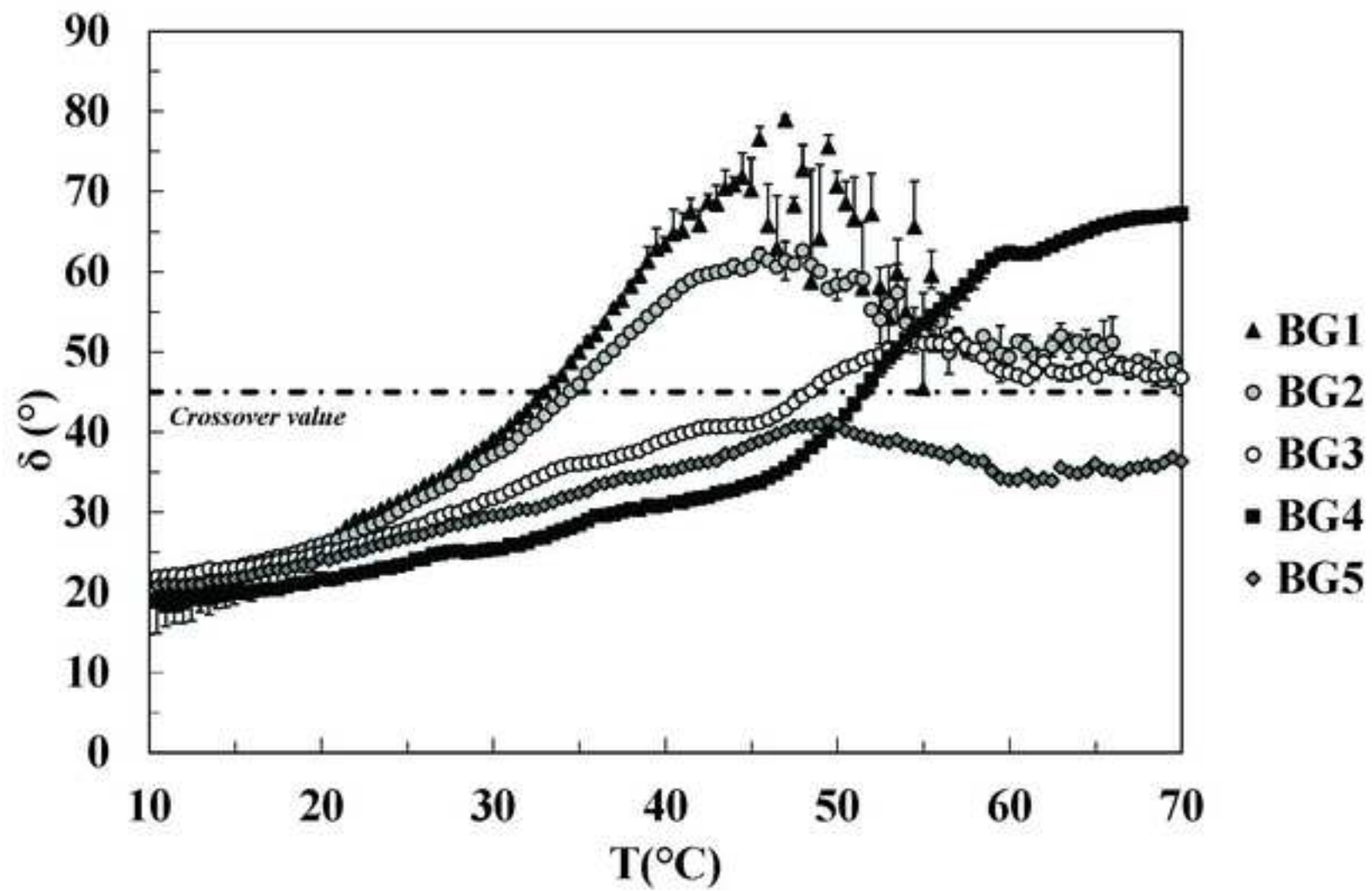


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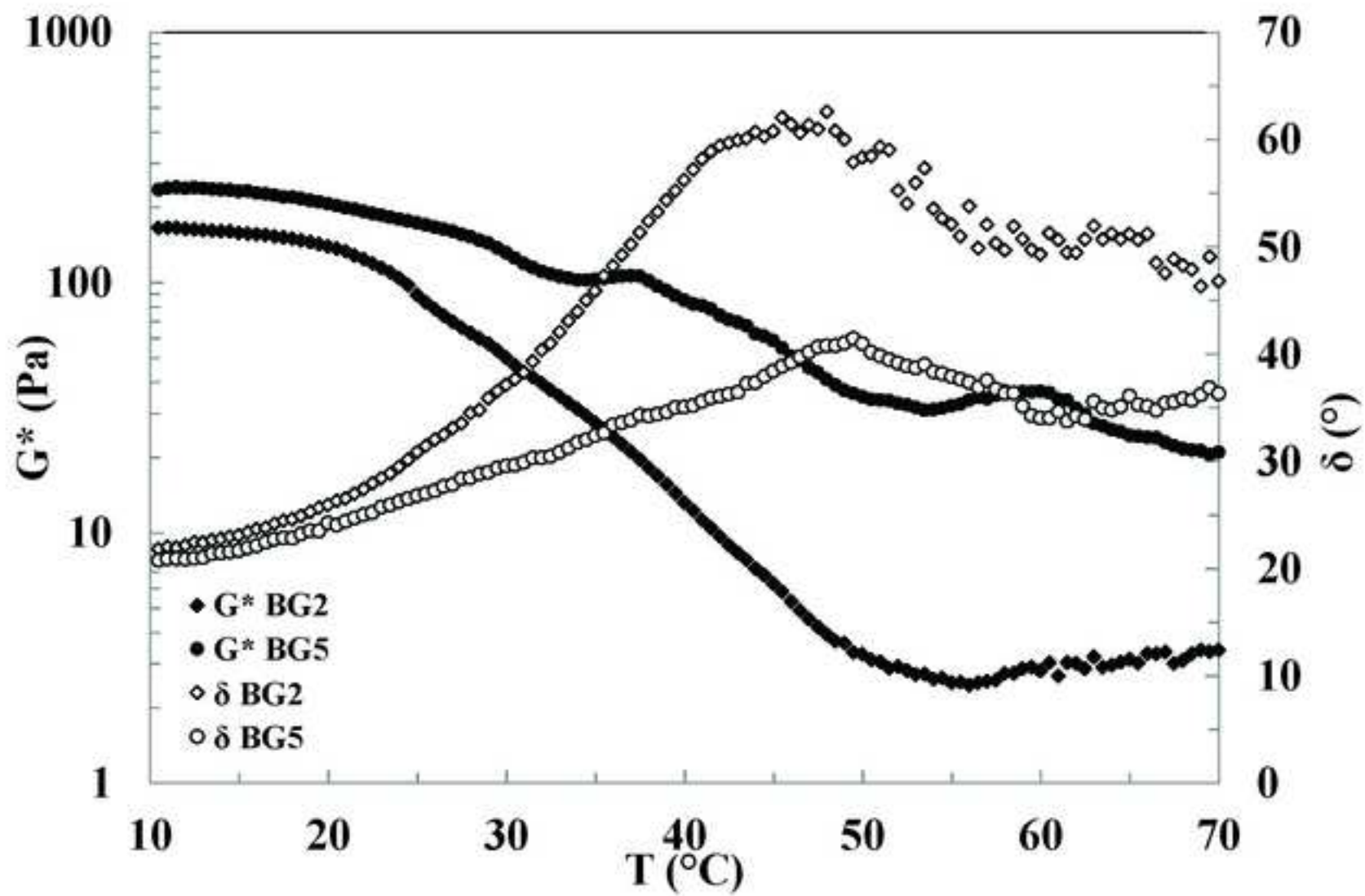


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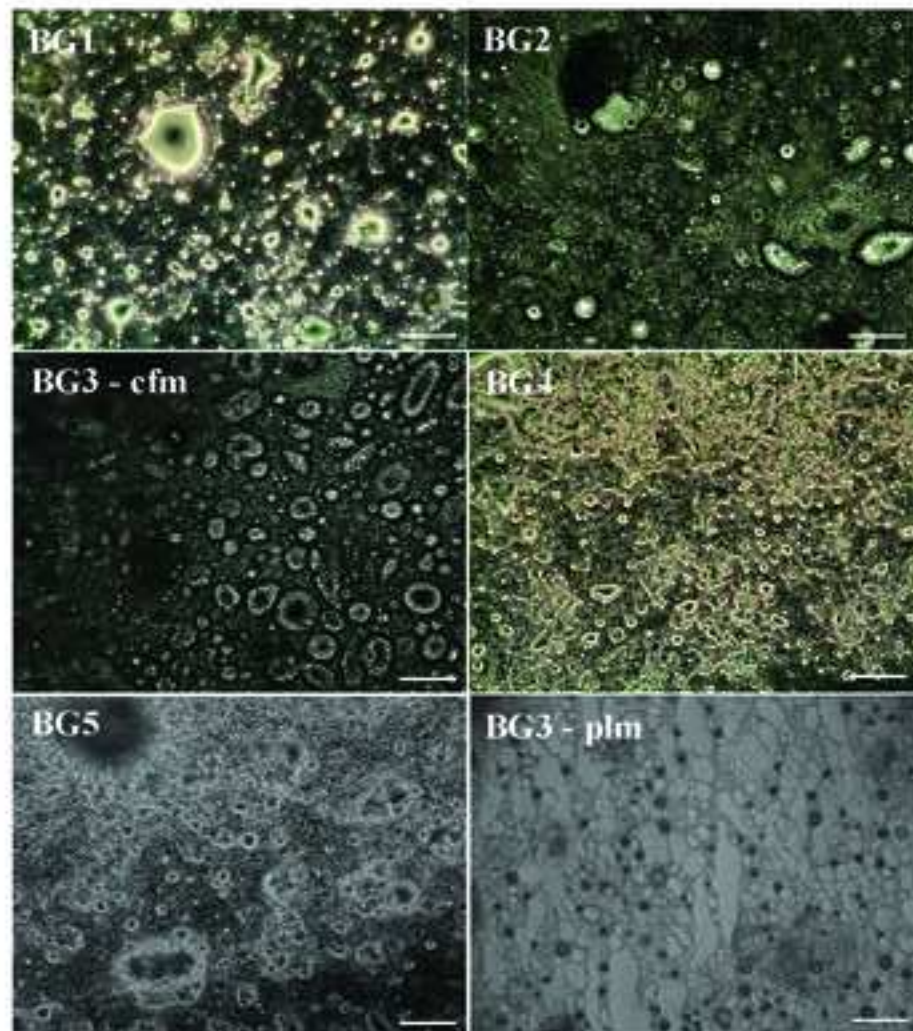


Table 1

Ingredients and properties	BG1 (%w/w)	BG2 (%w/w)	BG3 (%w/w)	BG4 (%w/w)	BG5 (%w/w)
Olive Oil	7.5	15	30	45	10
P	1.25	2.5	5	7.5	5
GS	1.25	2.5	5	7.5	5
Pectin	1.125	1	0.75	0.5	1
Glycerin	3.375	3	2.25	1.5	3
Buffer solution	85.5	76	57	38	76
Organogelators/oil ratio	0.333	0.333	0.333	0.333	1.0
Oil phase/aqueous phase	10:90	20:80	40:60	60:40	20:80
T_{cross} (°C)	33.0±0.2	34.5±0.1	48.3±0.2	51.5±0.3	-
\bar{d} (μm)	1.37±0.01	1.51±0.06	1.74±0.06	1.81±0.09	1.4±0.2
σ (μm)	0.34±0.01	0.39±0.06	0.81±0.07	0.82±0.03	0.4±0.2
η_0 (Pa·s)	(3.4±0.2)·10 ²	(7±0.4)·10 ²	(2.2±0.3)·10 ⁴	(5±1)·10 ⁵	(10.1±0.5)·10 ³
κ/κ_0 (-)	0.25	0.19	0.076	0.038	n.d.

Table 1

A rheological and microstructural characterisation of bigels for cosmetic and pharmaceutical uses

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Domenico Gabriele^a,

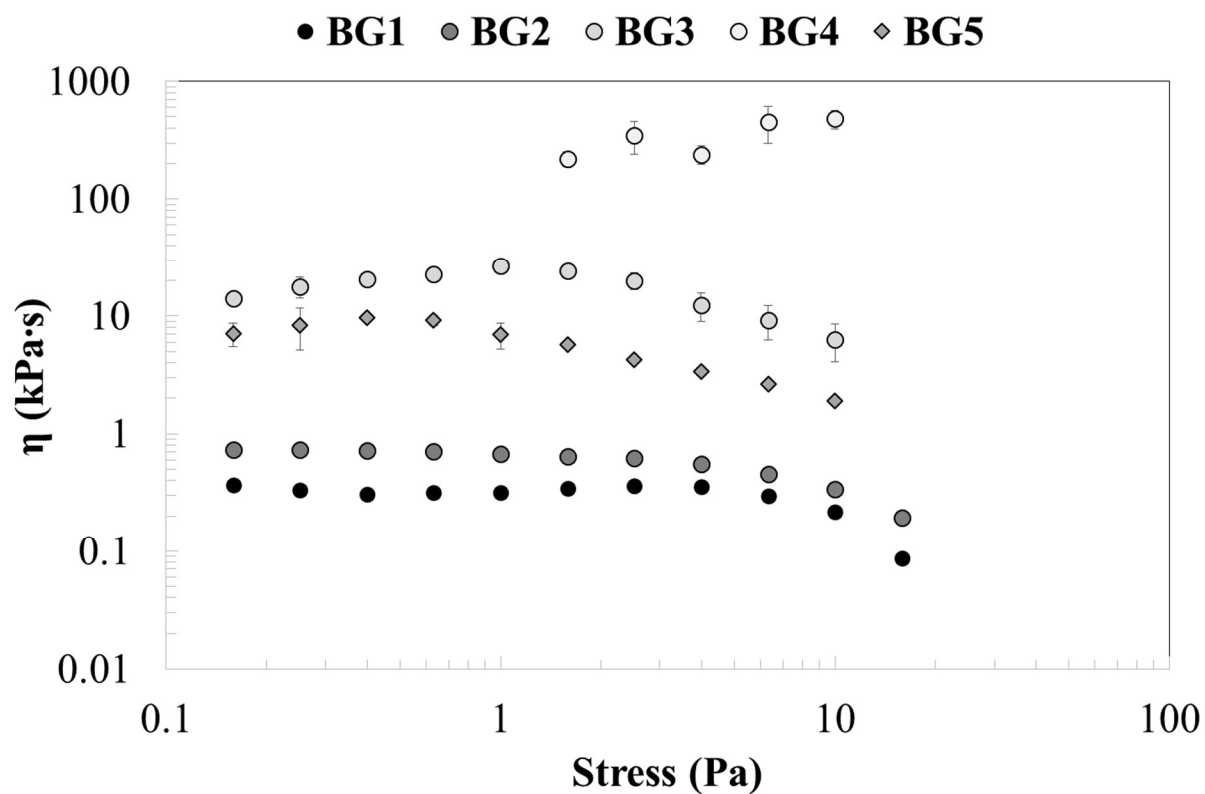


Fig. S1: Flow curve tests for bigels