Enhancing *trans*-Resveratrol loading capacity by forcing $W_1/O/W_2$ emulsions up to its colloidal stability limit

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Abstract

Trans-Resveratrol (3, 5, 4'-trihydroxystilbene) is a naturally occurring polyphenol easily oxidizable and extremely photosensitive with a short biological half-life that must be encapsulated to maintain its beneficial properties on the human body. The aim of this work is to increase the amount of resveratrol encapsulated using concentrated double water-in-oil-in-water ($W_1/O/W_2$) emulsions, making these systems more interesting as ingredient for functional food products formulations. The concentration of the inner emulsion (W_1/O) for several external (W_1O/W_2) ratios was optimized in terms of encapsulation efficiency (EE), colloidal stability and rheological behaviour. W_1/O emulsions formulated with ratios of 30/70 and 40/60 were used to obtain double emulsions (with ratios of 20/80 up to 80/20 of W_1O/W_2).

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Trans-Resveratrol EE increased up to 90% when the most concentrated double emulsions were prepared for both W_1/O ratios tested. The maximum resveratrol concentrations on double emulsions were 10.8 mg/L and 14.4 mg/L when 30/70 and 40/60 of W_1/O ratios were used, respectively. However, longer time stability was found for double high internal phase emulsions (W_1O/W_2) with a ratio of 30/70 of W_1/O.

The double emulsion with 80/20 W_1O/W_2 volumetric ratios together with 30/70 of W_1/O seems suitable to be used as ingredient for pharmaceutical and food devices/products due to its high colloidal stability, clearly pseudoplastic and elastic behaviour, high EE and large RSV carried capacity.

Keywords

trans-Resveratrol, Encapsulation, High internal phase double emulsions, rheology, stability

1. Introduction

Trans-Resveratrol is a polyphenol with antioxidant nature that seems to produce beneficial effects on human health in the fight against cancer, diabetes, neurodegeneration, cardiovascular disorders, inflammation, and other age-related pathologies what has greatly increased its applications in pharmaceutical and cosmetics industry [1–10].

However, *trans*-Resveratrol needs to be encapsulated because of its high instability and photosensitive character [11,12]. Several types of colloidal systems were used for resveratrol encapsulation such as nanovesicles (niosomes, liposomes, transfersomes) [13–16], nanoemulsions [17], water-in-oil-in-water ($W_1/O/W_2$) double emulsions [18–20], polymeric and solid lipid nanoparticles, lipospheres, cyclodextrins, polymeric microspheres, yeast cells carriers and calcium or zinc pectinate beads [21]. It was demonstrated that resveratrol oxidation and photodegradation was delayed when entrapped improving the stability and the shelf life of *trans*-resveratrol by protecting it against several degradation processes [22,23].

Double emulsions have a wide application field as in food, cosmetic, pharmaceutical and medical where they are frequently used as biocompounds carriers [20,24–33]. They are highly desirable as drug delivery systems, when the therapy requires repeated administration, via ingestion or injection, and for compounds with very short half-life [34,35]. The single administration of actives component through a double emulsion has shown prolonged release properties, avoiding alterations caused by the environment (e.g. oxidation, light, enzymatic degradation) or during food digestion [36,37]. In the case of hydrophilic bioactive compounds they are encapsulated in the dispersed phase (inner aqueous droplets) and the intermediate phase acts as a barrier from the external environment, increasing chemical stability and shelf life.

It has been reported in previous studies that the use of emulsions with a large amount of internal phase provides higher stability against creaming, remaining stable for longer time. Satisfactory results were obtained with concentrated double emulsions [19] used to encapsulate *trans*-Resveratrol. However, the total amount of *trans*-Resveratrol encapsulated was limited (6.2 mg/L) by its poor solubility in water, being necessary the addition of ethanol in the W_1 where the *trans*-Resveratrol is incorporated. In this previous study, it was reported the effect of increasing the ratio of the primary emulsion into the external aqueous phase (W_1O/W_2) but using a constant W_1/O ratio of 20/80 since this is the ratio optimized and used by other authors in order to avoid destabilization by coalescence of the inner water droplets [20,24,26–29,31,32,38–40]. There are also some studies in which the ratio of 30/70 was reported [33,41–43] and only a few have reported higher ratios, such us, 40/60 [44,45] and 50/50 [30,46]. The larger the amount of inner water phase, where the hydrophilic biocompounds of interest are entrapped, the larger the amount encapsulated, but it is necessary to stablish the colloidal stability limit of these thermodynamically unstable systems [19,47].

To our knowledge, the effect of concentrating both emulsions W_1/O and W_1O/W_2 simultaneously on the resulting colloidal stability and encapsulation efficiency (EE) has not been previously explored. Therefore, the aim of this work is to increase the amount of *trans*-Resveratrol encapsulated using concentrated double water-in-oil-in-water ($W_1/O/W_2$) emulsions by optimizing the concentration both of the primary and secondary emulsions, making these systems more interesting ingredients for functional food products formulations due to its high biocompound loading capacity.

The influence of the inner emulsion W_1/O ratio was studied for several external W_1O/W_2 ratios (from 20/80 to 80/20). The formulation was optimized in terms of EE and the total amount of *trans*-Resveratrol encapsulated on the final emulsion without sacrificing emulsion stability and appropriate rheological properties.

2. Materials and methods

2.1 Materials

Trans-Resveratrol ($C_{14}H_{12}O_3$), absolute ethanol and Tween 20 (HLB 16.7) were purchased from Sigma–Aldrich (USA). Miglyol® 812 (density 945 kg/m^3 at 20°C), which is a neutral oil formed by esters of caprylic and capric fatty acids and glycerol, was supplied by Sasol GmbH (Germany). Polyglycerol polyricinoleate (PGPR, HLB 3.0) was supplied by Brenntag AG (Germany). Sodium chloride was obtained from Panreac (Spain). Deionized water (Millipore Elix 5, Merck, Germany) was used for the preparation of both aqueous phases.

HPLC-grade methanol, acetonitrile, 2-propanol, and acetic acid were obtained from Sigma Aldrich (USA).

2.2. Methods

2.2.1 Water-in-oil (W_1/O) inner emulsion preparation

Trans-Resveratrol is barely soluble in water and highly soluble in ethanol, but its solubility in alcohol decreases as the carbon number of the alcohol increases [48]. Thus, a 20% ethanol (v/v) aqueous solution was used as the dispersed phase containing 50 mg/L of *trans*-Resveratrol.

It has been reported that the addition of electrolytes to the aqueous phase increases the W_1/O emulsion stability. It has been suggested that the presence of electrolytes lowers the attractive force between water droplets, decreasing the dielectric constant of the aqueous phase, therefore reducing collision frequency. NaCl was added being 0.1 M its concentration in the inner aqueous phase. This was carried out in all double emulsions to ensure W_1 droplet stability [49–51].

Miglyol 812 was used as the continuous phase (oil phase) containing PGPR as hydrophobic surfactant previously added, and easily dissolved by magnetic stirring for 5 min. PGPR is commonly used in food formulation and has been demonstrated to be highly effective at stabilizing W_1/O emulsions [51–53].

The PGPR/internal aqueous phase ratio was kept constant to ensure that the surface of the dispersed droplets (W_1) was always covered by the PGPR. A ratio of 0.4 (w/w) was selected in order to ensure emulsion stability regarding preliminary results [20].

Two W_1/O volumetric ratios were studied, 30/70 and 40/60, since experiments performed at higher ratios led to destabilization of the final double emulsions. Both phases were emulsified in glass vessels by high shear mixing (SilentCruser M Homogenizer, Heidolph, Germany) using a 6 mm dispersing tool at 15,000 rpm for 10 min. A total amount of 100 g of emulsion was prepared each time.

2.2.2. Water-in-oil-in-water ($W_1/O/W_2$) double emulsions preparation

The $W_1/O/W_2$ double emulsions were prepared by dispersing the W_1/O primary emulsion into the external aqueous phase (W_2) using several volumetric ratios of W_1O/W_2: 20/80, 30/70, 40/60, 50/50, 60/40, 70/30, and 80/20, respectively.

W_2 was a 2% (w/v) Tween 20 solution in 0.1 M NaCl. Tween 20 was added as an outer stabilizer and was dissolved by stirring for 30 min. NaCl was added to the W_2 phase in order to equilibrate the osmotic pressure between W_1 and W_2 in all emulsions (0.1 M NaCl).

Emulsification was carried out by mixing the continuous and dispersed phases with the SilentCruser M Homogenizer (Heidolph, Germany) at 5,000 rpm for 2 min.

The mixing conditions are considerably milder than in the case of simple emulsion preparation in order to preserve the integrity of the initial emulsion.

2.2.3. Emulsion characterization

A. Droplet size distribution

Emulsion droplet size distributions were obtained by the laser light scattering technique in a Mastersizer S long bench apparatus (Malvern Instruments, Ltd., UK).

The refractive index of the Miglyol 812 (1.54) was used for double emulsions droplet size measurements.

Samples were first diluted with deionized water to prevent multiple scattering effects. They were then circulated through the measuring zone using a Hydro SM small volume sample dispersion unit, following the manufacturer's recommendations for this type of emulsion.

Three replicates were performed for each emulsion and the results were reported as the typical droplet size distribution.

The size results are expressed in terms of equivalent spherical diameter, that is, the diameter of a sphere of the same volume as the measured particle. Emulsions usually show spherical drops.

B. Colloidal stability

Emulsion stability was measured by backscattering (BS) and transmission (TS) profiles in a Turbiscan apparatus (Formulaction, France). Emulsion samples were placed without dilution in the test cells. Transmitted and backscattered light was monitored as a function of time and cell height for 7 days at 30°C. TS and BS data were collected every 40 μm and given in percentage relative to the standard (suspension of monodisperse spheres in silicone oil) as a function of the sample height (in mm). These profiles build up a macroscopic fingerprint of the emulsion at a given time, providing useful information about changes in droplet size distribution, appearance of a creaming layer or a clarification front with time.

The Turbiscan Stability Index (TSI) is the sum of all the variations detected in the samples in terms of size and/or concentration, and is defined by equation (1):

$$TSI = \sum_i \frac{\sum_i |scan_i - scan_{i-1}|}{H} \quad (1)$$

where H is the total height of the cell at i interval time.

C. Visual inspection

Micrographs were used for emulsions visual inspection to confirm the droplet size obtained by laser light scattering. Micrographs of the emulsions were obtained with an Olympus BX50 light microscope (Olympus, Japan) with 10–100× magnification using UV–vis.

D. Rheology

159 The rheological tests were carried out with a Haake MARS II rotational rheometer
160 (ThermoFisher Scientific) with a Peltier unit to control the temperature. All the analyses were
161 carried out at 25±0.1°C and a plate/plate measuring system (PP60Ti) with a gap of 1 mm was
162 employed. Samples rested for at least 5 min previous to any measurement, allowing the
163 stresses induced during sample load to relax. Flow curves were carried out in CR mode from
164 0.01 to 500 s^{-1} Pa in 500 s. The Ostwald-de Waele model parameters were fitted to the
165 obtained data:

166 $$\tau = k\,\dot{\gamma}^{n} \qquad (2)$$

167 Where τ is the shear stress, $\dot{\gamma}$ the shear rate, *k* the consistency index and *n* the flow index.
168 Viscoelastic properties of the emulsions were assessed by means of frequency sweeps that
169 were carried out from 0.1 to 600 rad/s at a constant shear stress of 0.1 Pa.

170 *E. Determination of the initial encapsulation efficiency (EE)*

171 *Trans*-Resveratrol content in the external aqueous phase was determined by HPLC
172 chromatography (HP series 1100 chromatograph, Hewlett Packard, USA). The system was
173 equipped with a UV-vis absorbance detector HP G1315A or a fluorescence detector 1260
174 Infinity A (Agilent Technologies, USA).

175 The separation was performed with a Zorbax Eclipse Plus C18 reversed phase column, with a
176 particle size of 5 μm and 4.6 mm × 150 mm (Agilent Technologies, USA).

177 The mobile phase consisted of a mixture of (A) 100% milliQ-water and (B) 100% methanol with
178 gradient elution at a flow rate of 0.8 mL/min. The step gradient started with 80% mobile phase
179 (A) changing to 100% of mobile phase (B) in 5 minutes, maintaining it for another 10 min. The
180 mobile phase (B) was run for 2 min after each injection to prepare the column for the next run.
181 Separation was carried out at room temperature. A wavelength of 305 nm was used by the

182 UV-vis detector while the fluorescence detector was used at $\lambda_{excitation}$/ $\lambda_{emission}$ of 310/410 nm.
183 The column was cleaned after each group of samples by first running the mobile phase (A) for
184 20 min and a mobile phase (C) consisting of 50% acetonitrile, 25% milliQ-water, 25% 2-
185 propanol, and 0.01% acid acetic for 40 min at a flow rate of 0.25 mL/min. Finally, the column
186 was rinsed with 50% of the mobile phase (A) and 50% of the mobile phase (B) for another 20
187 min.

188 The external aqueous phases injected in the HPLC were previously separated by centrifugation
189 at 5000 rpm for 10 min, followed by filtration with a 0.22 μm polyvinylidene difluoride syringe
190 filter to remove all the oil phase that could be still present.

191 The recovery yield (R_y) of *trans*-Resveratrol after the centrifugation and filtration stages was
192 used to determine the amount of *trans*-Resveratrol that is transferred from the inner emulsion
193 (W_1/O) to the outer aqueous phase (W_2). For this purpose, an emulsion simulating the
194 situation of 100% transfer of W_1 phase into W_2 phase is prepared. An oil-in-water (O/W_2)
195 emulsion was prepared following the procedure described, and then W_1 phase was added,
196 simulating that all the components of W_1 were transferred to W_2. The *trans*-Resveratrol
197 concentration for this case is denominated the maximum concentration expected (C_0) in
198 equation 3. The *trans*-Resveratrol concentration measured in the separated aqueous phase
199 from the double emulsions prepared, analyzed by Reverse Phase HPLC is denominated
200 recovered concentration ($C_{recovered}$) in equation 3. The recovery yield, R_y, was calculated as:

201 $$R_y\ (\%) = \frac{C_{recovered}}{C_0} \cdot 100 \qquad (3)$$

202 EE of double emulsions was defined as the percentage of encapsulated *trans*-Resveratrol in W_1
203 that remained in the W_1/O primary emulsion after the second emulsification step. It was
204 calculated using the recovery yield defined in equation 3 as:

205 $$EE\ (\%) = 100\ - \frac{C_{recovered} \cdot 100}{C_0 \cdot R_y} \qquad (4)$$

206 At least three replicates of the analytical measurements were conducted for each sample and
207 the average values were taken.

208 ## 3. Results and discussion

209 The influence of the ratio of the W_1O/W_2 on final emulsion properties was studied in two
210 series of experiments. The ratio of W_1O/W_2 was varied from 20/80 to 80/20 using 10 %
211 increments. Each series was carried out using simple emulsions of ratio 30/70 and 40/60 of
212 W_1/O.

213 ### 3.1. Droplet size and visual inspection

214 Droplet size distributions for double emulsions with an internal phase concentration W_1/O of
215 30/70 are shown in Figure 1A.

216 **Figure 1**

217 Two well-defined peaks can be observed for each sample in both series. This bimodal
218 distribution has been observed in previous studies [19,20,54]. The first peak is due to the
219 formation of inverse PGPR micelles in the external aqueous phase W_2 [55].

220 Two trends can be clearly identified in the droplet size distribution. The main drop size
221 decreases with the W_1O/W_2 ratio. Double emulsions $D_{[4,3]}$ (volume weighted mean diameter)
222 decreased from 29.8 μm to 10.3 μm when W_1O/W_2 ratio increased from 20/80 to 80/20, being
223 the more concentrated emulsions the ones with narrower size distribution. The same trends
224 were already observed in previous works [19]. Figure 1A also shows that the most significant
225 changes take place close to the Ostwald critical concentration, being the ratios of W_1O/W_2,
226 70/30 and 80/20 the ones that presented narrower size distribution.

A similar trend was observed for double emulsions formulated with a 40/60 W_1/O ratio, as shown in Figure 1B regarding a decrease in $D_{[4,3]}$ from 41.8 μm to 7.5 μm for emulsions with an external ratio of 20/80 and 80/20, respectively.

The reduction on droplet size can be attributed to the increase of the internal emulsion viscosity due to their larger oil fraction. Similar results were observed when simple emulsions with oils of several viscosities were prepared, resulting that oil-in-water emulsions prepared with higher oil viscosity produced emulsions with smaller droplet size [47].

Moreover, for emulsions with ratio 40/60 (W_1/O), it can be observed at naked eye that emulsions results in a very low viscosity. According to Salager studies [56], the reduction in droplet size and the reduction in viscosity are the parameters that indicate the proximity to emulsion inversion point.

Figure S1 in supplementary material shows the optical microscopy images of the more concentrated double emulsions formulated with 30/70 and 40/60 ratios of W_1/O, respectively. The presence of inner water droplets (W_1) can be observed in all images confirming the presence of double $W_1/O/W_2$ emulsions being the oil droplets smaller and more packed as the W_1O/W_2 ratio increases. It can also be observed how the droplets lose their sphericity when they exceed the Ostwald critical concentration due to the high degree of packing (Fig. S1 EF) regarding also presence of some bigger droplets probably due to coalescence for emulsions formulated using W_1/O of 40/60 (Fig. S1 F). Similar results were reported by Gutiérrez et al. with concentrated simple emulsions [47].

3.2. Colloidal stability

Backscattering (BS) profiles from stability measurements for all double emulsions of both series of experiments are shown in Figures S2 and S3 of supplementary material corresponding to double emulsions formulated with W_1/O ratios of 30/70 and 40/60, respectively. Creaming

was produced by migration of the droplets to the upper part of the cell due to density differences between both phases. Migration of oils drops to the surface was observed in all BS profiles, being quicker for double emulsions with lower W_1O/W_2 ratios, as expected due to less hindrance produce by the presence of other drops on .creaming phenomenon was less important at high W_1O/W_2 ratios.

However, creaming phenomena due to drop migration is not only dependent on droplet density and W_1O/ W_2 ratios but also depends on droplet size. It is also important to realize that not all double emulsions have the same droplet size (Fig. 1) what also implies a clear influence on creaming phenomena. Modified Stokes law equation (including the internal phase volume fraction) [57] was used to calculate migration velocity (V), as it was reported in previous works (equation 5):

$$V = \frac{gd^2(\rho_w - \rho_o)}{18\mu\rho_w} \frac{(1-\phi)}{\left[1+\frac{4.6\phi}{(1-\phi)^3}\right]} \quad (5)$$

where d is the droplet diameter, ρ_w, ρ_o are the densities of the external phase and droplet respectively, μ is the continuous phase viscosity of the external phase and ϕ the internal phase volume fraction. Results are presented in Table S1 of supplementary material. For calculation, mean volume diameter, $D_{[4,3]}$, was used for all samples.

Table 1 shows that theoretically creaming phenomenon will appear sooner for emulsions prepared with low W_1O/W_2 ratio and will be more pronounced for those emulsions prepared with 40/60 W_1/O ratio. Excepting for more concentrated emulsions prepared with high W_1O/W_2 ratio (70/30 and 80/20) which according to equation 5 was expected to had higher migration velocities for emulsions formulated with an internal W_1/O ratio of 30/70 than for those prepared with 40/60.

Table 1

Table 1 also presents the clarification height measured after one week for all samples prepared. The larger the W_1O/W_2 ratio used, the lower the clarification height as it was expected, since there was a lower concentration of oil droplets to migrate

Surprisingly, emulsion with higher W_1O/W_2 ratio and W_1/O ratio of 40/60 did not follow the trend described, indicating that probably other destabilization phenomena could be taking place either escape of internal water droplets to the external aqueous phase or oil droplet coalescence. Observing backscattering profile of this sample (Figure S4 G) it was appreciated that a change of the backscattering value was at the middle of the sample, what is an indication that a variation in mean size is taking place (coalescence of oil droplets or swelling of W_1 drops).

Figure 2 shows the Turbiscan Stability Index (TSI) measured along time. Lower values of TSI imply higher emulsion stability as it reflects minor changes in the emulsion during ageing [58].

Figure 2

Trend observed is in good agreement with what has been aforementioned since either Figure 2A or Figure 2B show that emulsion stability increased with the W_1O/W_2 ratio for both inner emulsion ratios used (W_1/O). Similar trend was observed for simple and double emulsions in previous studies [19,47] and agree with the conclusions extracted from clarification heights measured and theoretical migration velocities. However, it was observed in Figure 2B the change on the trend for the more concentrated emulsions with a W_1/O ratio of 40/60, being TSI value higher for double emulsion with W_1O/W_2 ratio of 20/80 than for the emulsion with W_1O/W_2 ratio of 30/70.

As a general trend, TSI values after one week showed that the higher W_1/O ratio tested (40/60) produce less stable final double emulsions (higher TSI values) than those obtained with the lower W_1/O ratio tested (30/70)

From the stability results the optimum formulation was the double emulsion prepared with an W_1/O ratio of 30/70 and W_1O/W_2 ratio of 80/20.

3.3. Rheology

The effect of W_1/O to W_2 ratio on rheological behaviour of emulsions is shown in Figure 3. In addition, the viscoelastic properties of double emulsions have been assessed by means of frequency sweeps and results can be seen in Figure 4 and Figure 5.

Figure 3

It is remarkable that flow curves of double $W_1/O/W_2$ emulsions prepared with W_1/O ratios of 50/50 and 40/60 showed a deviation from linearity at a shear rate of 100 s^{-1} (Figure 3B). This singular behaviour has been previously reported in highly concentrated surfactant solutions [19,59,60] and it can be attributed to shear-induced disordering and entanglement of the micelles. Indeed, the deformation and rupture of the aggregates of the drops located in the upper part of the sample (due to the difference in density between the oily and aqueous phases) are responsible of this phenomenon.

Parameters of the Ostwald de Waele model obtained for double emulsions are shown in Table 2.

Table 2

The values obtained for the flow index (n) for double emulsions with a 30/70 W_1/ O ratio decreased with the W_1O/W_2 ratio. Additionally, the flow index was 1.098 for a 20/80 W_1O/W_2 ratio, which indicated a slightly dilatant behaviour, while the rest of the samples showed a pseudoplastic behaviour ($n < 1$). This property has also been underlined by other authors [61,62]. This pseudoplastic behaviour increased with the W_1O/W_2 ratio. Indeed, the flow index value varied between 0.8 and 0.9 (reflecting an almost Newtonian behaviour) for 30/70, 40/60

and 50/50 W_1O/W_2 ratios, but decreased to 0.4 for the emulsion with 80/20 ratio (indicating a clear pseudoplastic behaviour). *n* values for emulsions with a 40/60 of W_1/O ratio, showed a slightly dilatant behaviour for low W_1O/W_2 ratios (20/80 and 30/70) whereas the rest of samples exhibited a scarcely pseudoplastic character, showing an almost Newtonian behaviour (*n* was approximately 0.9). This dilatant behaviour, i.e., viscosity increases with applied shear stress, may be caused by the scape of water droplets from W_1 to W_2 at higher values of shear stress, which entails an increment in viscosity values.

Moreover, regarding viscosity values at 100 s^{-1} of shear rate for both double emulsions, 30/70 and 40/60, an increase is observed when the W_1O/W_2 ratio increases; this can be originated by the inner droplets scape from W_1 to W_2 due to coalescence phenomena. It also should be noticed the great difference observed between double emulsions in case of 80/20 ratio, viscosity value was almost 12 times higher in 30/70 emulsion in comparison to 40/60 one. This lower viscosity value seems to indicate higher instability in 40/60 emulsion, which was near to the inversion point, again probably due to the scape of water droplets from W_1 to W_2 [47].

The consistency index (*k*), parameter that gives an idea of the viscosity of a fluid, increased with W_1/O to W_2 ratio. Furthermore, *k* values for 30/70 to 60/40 W_1/O to W_2 ratios were in the same order of magnitude for both series of double emulsions prepared (30/70 and 40/60 W_1 to O ratios), whereas for 70/30 and 80/20 W_1/O to W_2 ratios, *k* values were much higher in the case of 30/70 W_1 to O ratio. It is important to point out that emulsion with 70/30 and 80/20 W_1/O to W_2 ratios prepared with a 40/60 W_1 to O ratio showed clear proximity to the inversion point and low viscosity.

The results of frequency sweeps performed in order to determine viscoelastic behaviour of the emulsions are shown in Figure 4.

Figure 4

345 Figure 4A and Figure 4B show that both moduli increased with frequency. From 20/80 up to
346 60/40 W_1O/W_2 ratios the viscous modulus (*G*") is higher than the elastic modulus (*G*') at low
347 frequencies, their values intersect at approximately 1 rad/s.

348 For higher W_1O/W_2 ratios (70/30 and 80/20), Fig. 4B, emulsions showed a more elastic
349 behaviour for all frequencies. The difference between the value of G' and G'' was more
350 pronounced for the case of 80/20 W_1O/W_2 ratio. For this last case, the elastic modulus was
351 practically constant for all frequencies, with a value of around 100 Pa, only increasing at very
352 high frequencies (near to 100 rad/s), which is in agreement with results obtained in a previous
353 work [19].

354 Viscous and elastic modulus for 40/60 W_1 to O ratio are shown in Figure 4C and Figure 4D.

355 The results were in general very similar to those shown in Figure 4A and Figure 4B, especially
356 for low W_1O/W_2 ratios represented in Figure 4C.

357 Emulsions with high W_1O/W_2 ratio shown in Figure 4D presented the same trend as shown in
358 Fig. 4B, but with values significantly lower. For the emulsions with W_1O/W_2 ratios of 60/40 and
359 70/30, both *G'* and *G"* moduli were almost three orders of magnitude lower than those of the
360 emulsions with W_1/O ratio of 30/70 and the same ratios of W_1O/W_2. Finally, for the 80/20
361 W_1O/W_2 ratio, the elastic modulus also remained practically constant throughout the
362 frequency sweep for the concentrated emulsion, although the value was one order of
363 magnitude lower than for the emulsions with W_1/O ratio of 30/70.

364 **3.4. Encapsulation efficiency (EE)**

365 Figure 5A shows the EE values for all emulsions prepared at different W_1/O and W_1O/W_2
366 ratios.

367 **Figure 5**

368 Resveratrol EE increased slowly with the W_1O/W_2 ratio from 20/80 to 50/50 for both series of
369 experiments (W_1/O ratio 30/70 and 40/60). However, in this range the emulsions with W_1/O
370 ratio of 30/70 had a considerably higher EE, from twice to three times with respect to the
371 emulsions with W_1/O ratio of 40/60. The sharpest increase in EE value occurs for the highest
372 W_1O/W_2 ratios for both series. Similar behaviour was observed in a previous work for
373 emulsions prepared using a 20/80 W_1/O volumetric ratio [19]. Therefore, these data have also
374 been plotted for easy comparison (Fig. 5A). Both series of 30/70 and 40/60 W_1/O ratios
375 showed the similar EE values for emulsions with W_1O/W_2 ratios from 60/40 to 80/20, reaching
376 a value of 90% of EE for the higher oil droplet concentration.

377 The maximum concentration of resveratrol entrapped was 10.8 mg/L for the emulsions
378 prepared using 30/70 of W_1/O and 14.4 mg/L for the one prepared using 40/60 of W_1/O
379 (Figure 5B). Observing encapsulated *trans*-Resveratrol concentration, similar value was
380 obtained for the following two emulsions: (i) ratios 30/70 (W_1/O) and 80/20 (W_1O/W_2) (ii)
381 ratios 40/60 (W_1/O) and 70/30 (W_1O/W_2) which were the more concentrated double
382 emulsions prepared with acceptable stability. Previous works reported EE for double
383 emulsions prepared using a volumetric ratio of W_1/O of 20/80 and a W_1O/W_2 ratio of 80/20
384 was 77.5% with a total encapsulated resveratrol concentration of 6.2 mg/L [19]. Studies with
385 different composition of the internal W_1 phase indicate a total encapsulated resveratrol
386 concentration between 0.5 and 388 mg/L [63] but to arise higher values to the ones presented
387 in this work higher amounts of ethanol were required what could be a disadvantage from the
388 final food application point of view.

389 From the data obtained in the present work and in the ones previously published [19] it can be
390 concluded that the amount of resveratrol encapsulated increases with the increase of the
391 internal phase concentration. These results were expected in the sense that more aqueous
392 drops were incorporated into the final emulsion formulated but also an increase in EE was

observed as the concentration of the internal phase increases making that the resveratrol emulsion capacity increases exponentially in all cases.

Moreover, analyzing in depth the variation of EE as W_1O/W_2 ratios increased for the three series of emulsions formulated with inner ratios of 20/80, 30/70 and 40/60, two different behaviors were observed. A nearly constant EE value was registered until W_1O/W_2 ratio of 60/40 for all ratios of W_1/O tested, regarding an EE increase as the W_1/O ratio increases both for 20/80 and 30/70 within this range. However, this is not the case for the double emulsions prepared with the W_1/O ratio of 40/60, probably due to the instability of this type of emulsions (as it is previously observed) what could produce the early escape of the W_1 drops to the external aqueous phase.

On the other hand, from W_1O/W_2 ratios of 60/40 to 80/20 EE increased exponentially for the three cases studied, observing then a synergic effect of the concentration of the aqueous droplets (W_1) and the oil droplets (W_1O). From these conclusions two equations could be used to predict *trans*-Resveratrol EE in double emulsions. One could be valid for double emulsions in which the external concentration (W_1O/W_2) does not exceed the value of 60/40 (equation 6) in which the only parameter will be the fraction of the W_1 drops (ϕ_w). Another equation (equation 7) could be valid for double emulsions with W_1O/W_2 ratios higher than 60/40, and will have two parameters ϕ_w and the fraction of W_1O drops (ϕ_{wo}).

$$EE = k\phi_w \quad (6)$$

$$EE = {\phi_w}^a x {\phi_{wo}}^b \quad (7)$$

where k, a and b will be the adjusting parameters.

4. Conclusions

The feasibility of preparing high concentrated double emulsions encapsulating *trans*-Resveratrol with a simple W_1/O emulsion of 30/70 W_1 to O ratio has been demonstrated, being 80/20 the maximum W_1/O to W_2 ratio of the final double emulsion $W_1/O/W_2$ formulated. When higher W_1/O ratio is used (40/60) the final concentration of the double emulsion (W_1O/W_2) could not exceed 30/70 with satisfactory stability and rheological results.

As a general trend, the colloidal stability versus creaming phenomena of formulated double emulsions increases within the W_1O/W_2 ratio. However, a compromise with the total concentration of water and oil drops on the final double emulsions should be taken into account in order to avoid water swelling droplets and oil droplets coalescence.

Rheological behaviour of emulsions is highly dependent on the internal phase emulsion concentration. Nearly Newtonian behaviour has been observed for diluted emulsions tending to become more pseudoplastic as the concentration of the secondary W_1O/W_2 emulsions increases. The effect of internal phase on flow properties were clearly observed for emulsion formulated with a W_1/O ratio of 30/70, in comparison to 40/60 ones, as it was indicated by n parameter values.

Regarding oscillatory measurements, in both cases, 20/80, 30/70, 40/60, 50/50 and 60/40 emulsions were predominantly viscous at low angular velocities, while over 1 rad/s they became mainly elastic, regarding similar viscoelastic behaviour for both double emulsions formulated using primary W_1/O emulsions with 30/70 and 40/60 ratios.

The increase of the concentration of the inner emulsion had a positive influence on the final EE value when the external double emulsion was also concentrated. However, a negative effect of the concentration of the internal emulsion on EE was observed when diluted external double emulsions were tested.

The maximum concentration of resveratrol entrapped were around 11 mg/L for the most concentrated emulsions with acceptable stability and rheological behaviour.

This study indicates that not only is important to prepare high internal phase double emulsions by increasing the concentration of the secondary W_1O/W_2 emulsions but also a small increase of the concentration of the primary W_1/O emulsions could duplicate the amount of biocompound encapsulated.

The concentrated double emulsions formulated at this study seemed to be appropriated to be used as ingredient for food fortification formulations for functional food preparation due to its high stability and high EE as well as its clearly pseudoplastic nature.

5. Acknowledgments

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6. References

[1] O. Vang, N. Ahmad, C.A. Baile, J.A. Baur, K. Brown, A. Csiszar, D.K. Das, D. Delmas, C. Gottfried, H.Y. Lin, Q.Y. Ma, P. Mukhopadhyay, N. Nalin, J.M. Pezzuto, T. Richard, Y. Shukla, Y.J. Surh, T. Szekeres, T. Szkudelski, T. Walle, J.M. Wu, What is new for an old molecule? Systematic review and recommendations on the use of resveratrol, PLoS One. 6 (2011). https://doi.org/10.1371/journal.pone.0019881.

[2] T. Yang, L. Wang, M. Zhu, L. Zhang, L. Yan, Properties and molecular mechanisms of resveratrol: a review, Die Pharm. - An Int. J. Pharm. Sci. 70 (2015) 501–506. https://doi.org/10.1691/ph.2015.5571.

[3] G.T. Diaz-Gerevini, G. Repossi, A. Dain, M.C. Tarres, U.N. Das, A.R. Eynard, Beneficial action of resveratrol: How and why?, Nutrition. 32 (2016) 174–178. https://doi.org/10.1016/j.nut.2015.08.017.

[4] G. Murtaza, U. Latif, M. Najam-Ul-Haq, A. Sajjad, S. Karim, M. Akhtar, I. Hussain, Resveratrol: An active natural compound in red wines for health, J. Food Drug Anal. 21 (2013). https://doi.org/10.6227/jfda.2013210101.

[5] M.G. Novelle, D. Wahl, C. Diéguez, M. Bernier, R. De Cabo, Resveratrol supplementation: Where are we now and where should we go?, Ageing Res. Rev. 21 (2015) 1–15. https://doi.org/10.1016/j.arr.2015.01.002.

[6] J. Ruivo, C. Francisco, R. Oliveira, A. Figueiras, The main potentialities of resveratrol for drug delivery systems, Brazilian J. Pharm. Sci. 51 (2015) 499–514. https://doi.org/10.1590/S1984-82502015000300002.

[7] P. Saiko, A. Szakmary, W. Jaeger, T. Szekeres, Resveratrol and its analogs: Defense against cancer, coronary disease and neurodegenerative maladies or just a fad?, Mutat. Res. - Rev. Mutat. Res. 658 (2008) 68–94. https://doi.org/10.1016/j.mrrev.2007.08.004.

[8] X. Yang, X. Li, J. Ren, From French Paradox to Cancer Treatment: Anti-cancer Activities and Mechanisms of Resveratrol, Anticancer. Agents Med. Chem. 14 (2014) 806–825. https://doi.org/10.2174/1871520614666140521121722.

[9] N. Machado, M. Fernández, D. Díaz, Recent Strategies in Resveratrol Delivery Systems, Chempluschem. 84 (2019) 951–973. https://doi.org/10.1002/cplu.201900267.

[10] J. Betz, Resveratrol and its Effects on Human Health and Longevity - Myth or Miracle?, Taichung, Taiwan, 2011.

[11] M. Vian, V. Tomao, S. Gallet, P. Coulomb, J. Lacombe, Simple and rapid method for cis-

484 and trans-resveratrol and piceid isomers determination in wine by high-performance
485 liquid chromatography using Chromolith columns, J. Chromatogr. A. 1085 (2005) 224–
486 229. https://doi.org/10.1016/j.chroma.2005.05.083.

487 [12] C.G. Silva, J. Monteiro, R.R.N. Marques, A.M.T. Silva, C. Martínez, M. Canle L., J.L. Faria,
488 Photochemical and photocatalytic degradation of trans-resveratrol, Photochem.
489 Photobiol. Sci. 12 (2013) 638–644. https://doi.org/10.1039/c2pp25239b.

490 [13] D. Pando, M. Beltrán, I. Gerone, M. Matos, C. Pazos, Resveratrol entrapped niosomes as
491 yoghurt additive, Food Chem. 170 (2015) 281–287.
492 https://doi.org/10.1016/j.foodchem.2014.08.082.

493 [14] D. Pando, C. Caddeo, M. Manconi, A. Fadda, C. Pazos, Nanodesign of olein vesicles for
494 the topical delivery of the antioxidant resveratrol, J. Pharm. Pharmacol. 65 (2013)
495 1158–1167. https://doi.org/10.1111/jphp.12093.

496 [15] D. Pando, G. Gutiérrez, C. Pazos, Preparation and characterization of niosomes
497 containing resveratrol, J. Food Eng. 117 (2013) 227–234.
498 https://doi.org/10.1016/j.jfoodeng.2013.02.020.

499 [16] E. Soo, S. Thakur, Z. Qu, S. Jambhrunkar, H.S. Parekh, A. Popat, Enhancing delivery and
500 cytotoxicity of resveratrol through a dual nanoencapsulation approach, J. Colloid
501 Interface Sci. 462 (2016) 368–374. https://doi.org/10.1016/j.jcis.2015.10.022.

502 [17] G. Davidov-Pardo, D.J. McClements, Resveratrol encapsulation: Designing delivery
503 systems to overcome solubility, stability and bioavailability issues, Trends Food Sci.
504 Technol. 38 (2014) 88–103. https://doi.org/10.1016/j.tifs.2014.05.003.

505 [18] Y. Hemar, L.J. Cheng, C.M. Oliver, L. Sanguansri, M. Augustin, Encapsulation of
506 resveratrol using Water-in-Oil-in-Water double emulsions, Food Biophys. 5 (2010) 120–

127. https://doi.org/10.1007/s11483-010-9152-5.

[19] M. Matos, G. Gutiérrez, L. Martínez-Rey, O. Iglesias, C. Pazos, Encapsulation of resveratrol using food-grade concentrated double emulsions : Emulsion characterization and rheological behaviour, J. Food Eng. 226 (2018) 73–81. https://doi.org/10.1016/j.jfoodeng.2018.01.007.

[20] M. Matos, G. Gutiérrez, J. Coca, C. Pazos, Preparation of water-in-oil-in-water (W1/O/W2) double emulsions containing trans-resveratrol, Colloids Surfaces A Physicochem. Eng. Asp. 442 (2014) 69–79. https://doi.org/10.1016/j.colsurfa.2013.05.065.

[21] A.R. Neves, M. Lucio, J.L.C. Lima, S. Reis, Resveratrol in Medicinal Chemistry: A Critical Review of its Pharmacokinetics, Drug-Delivery, and Membrane Interactions, Curr. Med. Chem. 19 (2012) 1663–1681. https://doi.org/10.2174/092986712799945085.

[22] T. Loftsson, M.E. Brewster, Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization, J. Pharm. Sci. 85 (1996) 1017–1025. https://doi.org/10.1021/js950534b.

[23] S. Chen, Y. Han, L. Jian, W. Liao, Y. Zhang, Y. Gao, Fabrication, characterization, physicochemical stability of zein-chitosan nanocomplex for co-encapsulating curcumin and resveratrol, Carbohydr. Polym. 236 (2020) 116090. https://doi.org/10.1016/j.carbpol.2020.116090.

[24] N. Kheynoor, S.M.H. Hosseini, G.H. Yousefi, H. Hashemi Gahruie, G.R. Mesbahi, Encapsulation of vitamin C in a rebaudioside-sweetened model beverage using water in oil in water double emulsions, Food Sci. Technol. 96 (2018) 419–425. https://doi.org/10.1016/j.lwt.2018.05.066.

[25] M. Matos, G. Gutiérrez, O. Iglesias, J. Coca, C. Pazos, Characterization, stability and rheology of highly concentrated monodisperse emulsions containing lutein, Food Hydrocoll. 49 (2015) 156–163. https://doi.org/10.1016/j.foodhyd.2015.03.021.

[26] H.J. Giroux, G. Robitaille, M. Britten, Controlled release of casein-derived peptides in the gastrointestinal environment by encapsulation in water-in-oil-in-water double emulsions, LWT - Food Sci. Technol. 69 (2016) 225–232. https://doi.org/10.1016/j.lwt.2016.01.050.

[27] L. Flaiz, M. Freire, S. Cofrades, R. Mateos, J. Weiss, F. Jiménez-Colmenero, R. Bou, Comparison of simple, double and gelled double emulsions as hydroxytyrosol and n-3 fatty acid delivery systems, Food Chem. 213 (2016) 49–57. https://doi.org/10.1016/j.foodchem.2016.06.005.

[28] J. O'Regan, D.M. Mulvihill, Sodium caseinate-maltodextrin conjugate stabilized double emulsions: Encapsulation and stability, Food Res. Int. 43 (2010) 224–231. https://doi.org/10.1016/j.foodres.2009.09.031.

[29] V. Eisinaite, D. Juraite, K. Schroën, D. Leskauskaite, Food-grade double emulsions as effective fat replacers in meat systems, J. Food Eng. 213 (2017) 54–59. https://doi.org/10.1016/j.jfoodeng.2017.05.022.

[30] A.K. Jena, A.K. Nayak, A. De, D. Mitra, A. Samanta, Development of lamivudine containing multiple emulsions stabilized by gum odina, Futur. J. Pharm. Sci. 4 (2018) 71–79. https://doi.org/10.1016/j.fjps.2017.10.002.

[31] V. Evageliou, E. Panagopoulou, I. Mandala, Encapsulation of EGCG and esterified EGCG derivatives in double emulsions containing Whey Protein Isolate, Bacterial Cellulose and salt, Food Chem. 281 (2019) 171–177. https://doi.org/10.1016/j.foodchem.2018.12.105.

554 [32] H.J. Giroux, S. Constantineau, P. Fustier, C.P. Champagne, D. St-Gelais, M. Lacroix, M.
555 Britten, Cheese fortification using water-in-oil-in-water double emulsions as carrier for
556 water soluble nutrients, Int. Dairy J. 29 (2013) 107–114.
557 https://doi.org/10.1016/j.idairyj.2012.10.009.

558 [33] D.J. Pimentel-González, R.G. Campos-Montiel, C. Lobato-Calleros, R. Pedroza-Islas, E.J.
559 Vernon-Carter, Encapsulation of Lactobacillus rhamnosus in double emulsions
560 formulated with sweet whey as emulsifier and survival in simulated gastrointestinal
561 conditions, Food Res. Int. 42 (2009) 292–297.
562 https://doi.org/10.1016/j.foodres.2008.12.002.

563 [34] P.P. Constantinides, Lipid Microemulsions for Improving Drug Dissolution and Oral
564 Absorption: Physical and Biopharmaceutical Aspects, Pharm. Res. 12 (1995) 1561–1572.
565 https://doi.org/10.1023/a:1016268311867.

566 [35] P. Constantinides, G. Welzel, H. Ellens, P. Smith, S. Sturgis, S. Yiv, A. Owen, Water-in-oil
567 microemulsions containing medium-chain fatty acids/salts: formulation and intestinal
568 absorption enhancement evaluation, Pharm. Res. 13 (1996) 210–215.
569 https://doi.org/10.1023/a:1016030812272.

570 [36] M.J. Rosen, Surfactants and Interfacial Phenomena, Third edit, Wiley Interscience,
571 Hoboken, USA, 2004.

572 [37] A. Aserin, Multiple Emulsion: Technology and Applications, Wiley Interscience,
573 Hoboken, USA, 2008.

574 [38] I. Scherze, R. Knöfel, G. Muschiolik, Automated image analysis as a control tool for
575 multiple emulsions, Food Hydrocoll. 19 (2005) 617–624.
576 https://doi.org/10.1016/j.foodhyd.2004.10.029.

[39] S. Mun, Y. Choi, J.Y. Shim, K.H. Park, Y.R. Kim, Effects of enzymatically modified starch on the encapsulation efficiency and stability of water-in-oil-in-water emulsions, Food Chem. 128 (2011) 266–275. https://doi.org/10.1016/j.foodchem.2011.03.014.

[40] S. Cofrades, J.A. Santos-López, M. Freire, J. Benedí, F.J. Sánchez-Muniz, F. Jiménez-Colmenero, Oxidative stability of meat systems made with W1/O/W2 emulsions prepared with hydroxytyrosol and chia oil as lipid phase, LWT - Food Sci. Technol. 59 (2014) 941–947. https://doi.org/10.1016/j.lwt.2014.06.051.

[41] R. Berendsen, C. Güell, M. Ferrando, A procyanidin-rich extract encapsulated in water-in-oil-in-water emulsions produced by premix membrane emulsification, Food Hydrocoll. 43 (2015) 636–648. https://doi.org/10.1016/j.foodhyd.2014.07.023.

[42] M. Shima, Y. Kobayashi, T. Fujii, M. Tanaka, Y. Kimura, S. Adachi, R. Matsuno, Preparation of fine W/O/W emulsion through membrane filtration of coarse W/O/W emulsion and disappearance of the inclusion of outer phase solution, Food Hydrocoll. 18 (2004) 61–70. https://doi.org/10.1016/S0268-005X(03)00042-0.

[43] A. Pawlik, P.W. Cox, I.T. Norton, Food grade duplex emulsions designed and stabilised with different osmotic pressures, J. Colloid Interface Sci. 352 (2010) 59–67. https://doi.org/10.1016/j.jcis.2010.08.049.

[44] M. Bonnet, M. Cansell, A. Berkaoui, M.H. Ropers, M. Anton, F. Leal-Calderon, Release rate profiles of magnesium from multiple W/O/W emulsions, Food Hydrocoll. 23 (2009) 92–101. https://doi.org/10.1016/j.foodhyd.2007.11.016.

[45] S.Y. Tang, M. Sivakumar, B. Nashiru, Impact of osmotic pressure and gelling in the generation of highly stable single core water-in-oil-in-water (W/O/W) nano multiple emulsions of aspirin assisted by two-stage ultrasonic cavitational emulsification, Colloids Surfaces B Biointerfaces. 102 (2013) 653–658.

https://doi.org/10.1016/j.colsurfb.2012.08.036.

[46] B.L. Rivas, M. Palencia, Removal-concentration of pollutant metal-ions by water-soluble polymers in conjunction with double emulsion systems: A new hybrid method of membrane-based separation, Sep. Purif. Technol. 81 (2011) 435–443. https://doi.org/10.1016/j.seppur.2011.08.021.

[47] G. Gutiérrez, M. Matos, J.M. Benito, J. Coca, C. Pazos, Preparation of HIPEs with controlled droplet size containing lutein, Colloids Surfaces A Physicochem. Eng. Asp. 442 (2014) 111–122. https://doi.org/10.1016/j.colsurfa.2013.05.077.

[48] X. Sun, B. Peng, W. Yan, Measurement and correlation of solubility of trans-resveratrol in 11 solvents at T = (278.2, 288.2, 298.2, 308.2, and 318.2) K, J. Chem. Thermodyn. 40 (2008) 735–738. https://doi.org/10.1016/j.jct.2007.10.006.

[49] S. Frasch-Melnik, F. Spyropoulos, I.T. Norton, W1/O/W2 double emulsions stabilised by fat crystals - Formulation, stability and salt release, J. Colloid Interface Sci. 350 (2010) 178–185. https://doi.org/10.1016/j.jcis.2010.06.039.

[50] J. Jiang, Z. Mei, J. Xu, D. Sun, Effect of inorganic electrolytes on the formation and the stability of water-in-oil (W/O) emulsions, Colloids Surfaces A Physicochem. Eng. Asp. 429 (2013) 82–90. https://doi.org/10.1016/j.colsurfa.2013.03.039.

[51] A.L. Márquez, A. Medrano, L.A. Panizzolo, J.R. Wagner, Effect of calcium salts and surfactant concentration on the stability of water-in-oil (w / o) emulsions prepared with polyglycerol polyricinoleate, J. Colloid Interface Sci. 341 (2010) 101–108. https://doi.org/10.1016/j.jcis.2009.09.020.

[52] R. Wilson, B. Van Schie, D. Howes, Overview of the preparation, use and biological studies on polyglycerol polyricinoleate (PGPR)., Food Chem. Toxicol. 36 (1998) 711–718.

https://doi.org/10.1016/s0278-6915(98)00057-x.

[53] F. Wolf, K. Koehler, H. Schuchmann, Stabilization of water droplets in oil with PGPR for use in oral and dermal applications, J. Food Process Eng. 36 (2013) 276–283. https://doi.org/10.1111/j.1745-4530.2012.00688.x.

[54] Y. Hemar, L. Cheng, C. Oliver, L. Sanguansri, M. Augustin, Encapsulation of Resveratrol Using Water-in-Oil-in-Water Double Emulsions, Food Biophys. 5 (2010) 120–127.

[55] M. Matos, G. Gutiérrez, O. Iglesias, J. Coca, C. Pazos, Enhancing encapsulation efficiency of food-grade double emulsions containing resveratrol or vitamin B12 by membrane emulsification, J. Food Eng. 166 (2015) 212–220. https://doi.org/10.1016/j.jfoodeng.2015.06.002.

[56] J.-L. Salager, L. Marquez, A. Peña, M. Rondón, F. Silva, E. Tyrode, Current Phenomenological Know-How and Modeling of Emulsion Inversion, Ind. Eng. Chem. Res. 39 (2000) 2665–2676. https://doi.org/10.1021/ie990778x.

[57] P. Snabre, P. Mills, Settling of a suspension of hard particles, Europhys. Lett. 25 (1994) 651–656.

[58] M. Matos, A. Marefati, G. Gutiérrez, M. Wahlgren, M. Rayner, Comparative Emulsifying Properties of Octenyl Succinic Anhydride (OSA) -Modified Starch : Granular Form vs Dissolved State, PLoS One. 11 (2016) 1–16. https://doi.org/10.1371/journal.pone.0160140.

[59] J.-F. Berret, Transient Rheology of Wormlike Micelles, Langmuir1. 13 (997) 2227–2234.

[60] R. Pal, Rheological behaviour of concentrated surfactant solutions and emulsions, Colloids and Surfaces. 64 (1992) 207–215. https://doi.org/10.1016/0166-6622(92)80101-7.

[61] I. Castangia, M.L. Manca, P. Matricardi, C. Sinico, S. Lampis, X. Fernàndez-Busquets, A.M. Fadda, M. Manconi, Effect of diclofenac and glycol intercalation on structural assembly of phospholipid lamellar vesicles, Int. J. Pharm. 456 (2013). https://doi.org/10.1016/j.ijpharm.2013.08.034.

[62] M.L. Manca, M. Manconi, A.M. Falchi, I. Castangia, D. Valenti, S. Lampis, A.M. Fadda, Close-packed vesicles for diclofenac skin delivery and fibroblast targeting, Colloids Surfaces B Biointerfaces. 111 (2013) 609–617. https://doi.org/10.1016/j.colsurfb.2013.07.014.

[63] J. Wang, A. Shi, D. Agyei, Q. Wang, Formulation of water-in-oil-in-water (W/O/W) emulsions containing trans-resveratrol, RSC Adv. 7 (2017) 35917–35927. https://doi.org/10.1039/c7ra05945k.

660 **7. Figure Captions**

661 Figure 1. Droplet size distribution of the double $W_1/O/W_2$ emulsions formulated with different
662 of W_1/O to W_2 ratios using 30/70 (A) and 40/60 (B) W_1 to O ratios

663 Figure 2. Evolution of TSI values for double emulsions formulated with different of W_1/O to W_2
664 ratios using 30/70 (A) and 40/60 (B) W_1 to O ratios

665 Figure 3. Flow curves obtained for the double emulsions $W_1/O/W_2$ at different W_1/O to
666 W_2ratios: (A) 20/80 and 30/70; (B) 40/60 and 50/50; (C) 60/40; (D) 70/30 and 80/20

667 Figure 4. Elastic modulus (G ') and viscous modulus (G ") versus frequency for double
668 emulsions $W_1/O/W_2$ with 30/70 W_1 to O ratio formulated for different W_1/O to W_2
669 ratios: (A) 20/80, 30/70, 40/60 and 50/50; (B) 60/40, 70/30 and 80/20; and for double
670 emulsions $W_1/O/W_2$ with 40/60 W_1 to O ratio formulated for different W_1/O to W_2
671 ratios: (C) 20/80, 30/70, 40/60 and 50/50; (D) 60/40, 70/30 and 80/20

672 Figure 5. Comparison between the double $W_1/O/W_2$ emulsions formulated with different of
673 W_1/O *to* W_2 ratios using 20/80, 30/70 and 40/60 W_1 to O ratios: A) the encapsulation
674 efficiencies (EE) and (B) encapsulated concentration of *trans*-Resveratrol on final
675 emulsion in mg/L

***Declaration of Interest Statement**

Declaration of interests

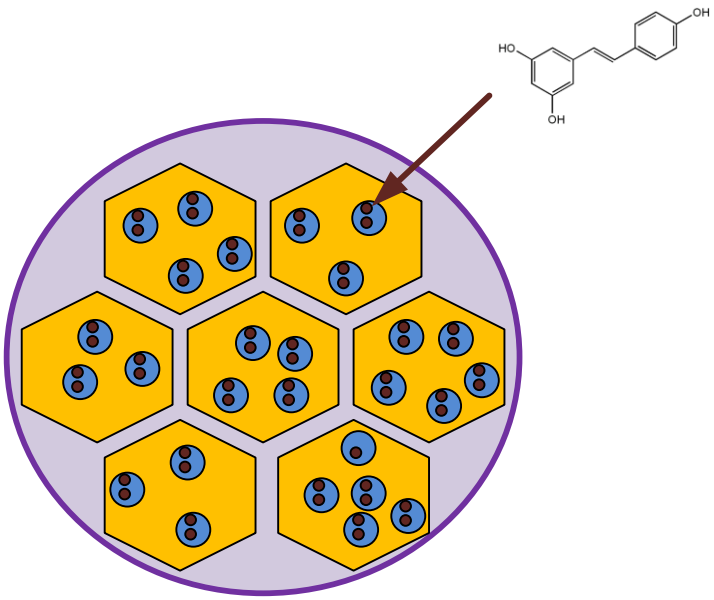
☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Resveratrol was encapsulated in water-in-oil-in water concentrated double emulsions
- Both the primary W_1/O and the final loaded $W_1/O/W_2$ emulsions were concentrated
- Resveratrol encapsulation efficiency (EE) was determined by RP-HPLC method
- Maximum concentration rate was optimized in terms of colloidal stability and EE
- The double emulsions were prepared by mechanical agitation using a two-step process

Graphical Abstract



Table(s) 1

Table 1. Theoretical migration velocities of oil drops to the sample surface and experimental clarification height

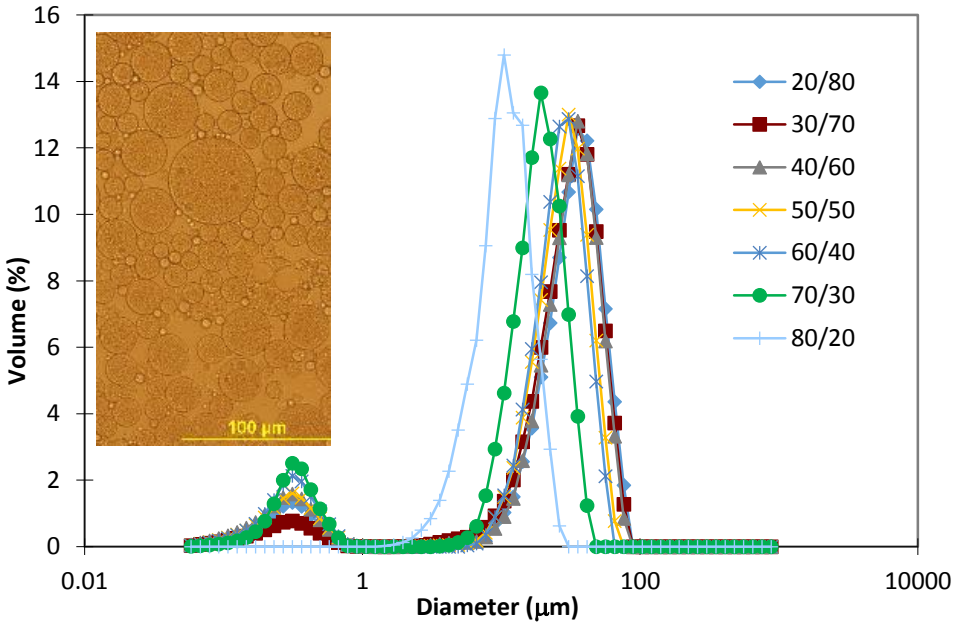
Ratio W_1O/W_2 emulsion	Experimental clarification height (mm)	Theoretical Migration velocity (m/s)	Experimental clarification height (mm)	Theoretical Migration velocity (m/s)
	Ratio W_1/O 30/70		Ratio W_1/O 40/60	
20/80	31.5	$6.2 \cdot 10^{-9}$	32.0	$1.2 \cdot 10^{-8}$
30/70	23.9	$2.9 \cdot 10^{-9}$	27.5	$6.7 \cdot 10^{-9}$
40/60	23.5	$1.2 \cdot 10^{-9}$	22.4	$2.9 \cdot 10^{-10}$
50/50	20.1	$3.7 \cdot 10^{-10}$	19.9	$6.9 \cdot 10^{-10}$
60/40	13.0	$1.1 \cdot 10^{-10}$	16.2	$1.4 \cdot 10^{-10}$
70/30	6.8	$1.5 \cdot 10^{-11}$	7.2	$1.1 \cdot 10^{-11}$
80/20	5.5	$1.1 \cdot 10^{-12}$	10.1	$5.9 \cdot 10^{-13}$

Table(s) 2

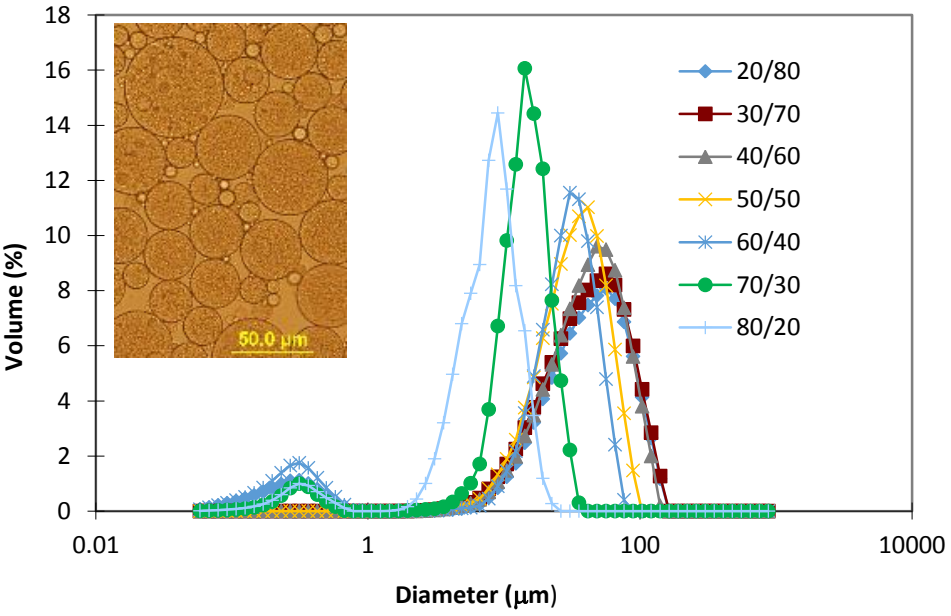
Table 2. Values of the parameters applying the rheological model of Ostwald-de Waele for each of the formulated double emulsions, and viscosity at 100 s^{-1}

Double emulsions ratio W_1/O to W_2	Internal phase W_1 to O: 30/70			Internal phase W_1 to O: 40/60		
	k	n	Viscosity (cP)	k	n	Viscosity (cP)
20/80	0.001	1.098	2.258	0.001	1.192	1.436
30/70	0.008	0.873	5.865	0.001	1.082	2.860
40/60	0.016	0.847	9.132	0.008	0.890	8.152
50/50	0.022	0.888	12.900	0.018	0.888	12.175
60/40	0.059	0.816	22.755	0.031	0.928	20.710
70/30	0.240	0.668	55.580	0.017	0.902	16.010
80/20	4.590	0.415	267.050	0.033	0.907	22.855

Figure(s) 1



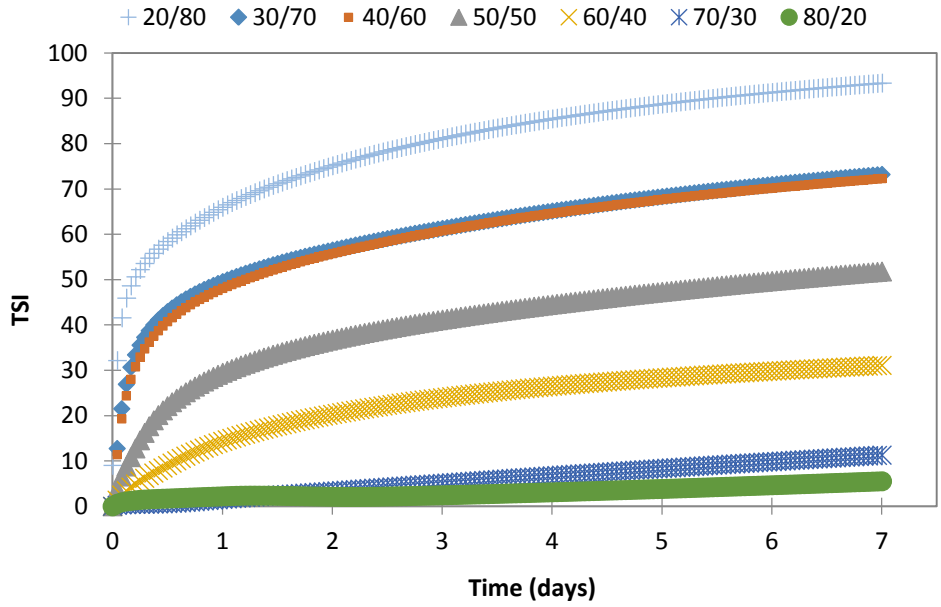
(A)



(B)

Figure 1

Figure(s) 2



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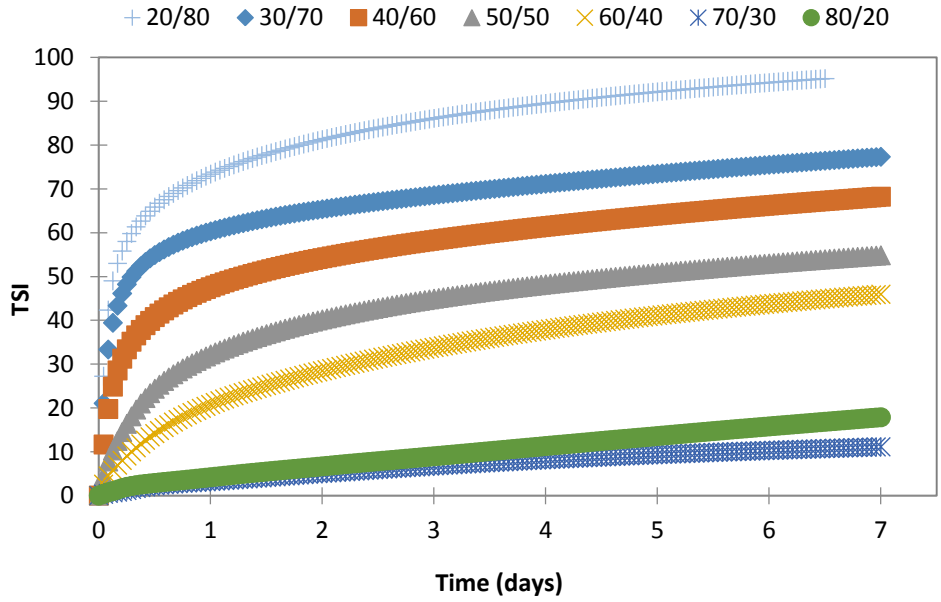
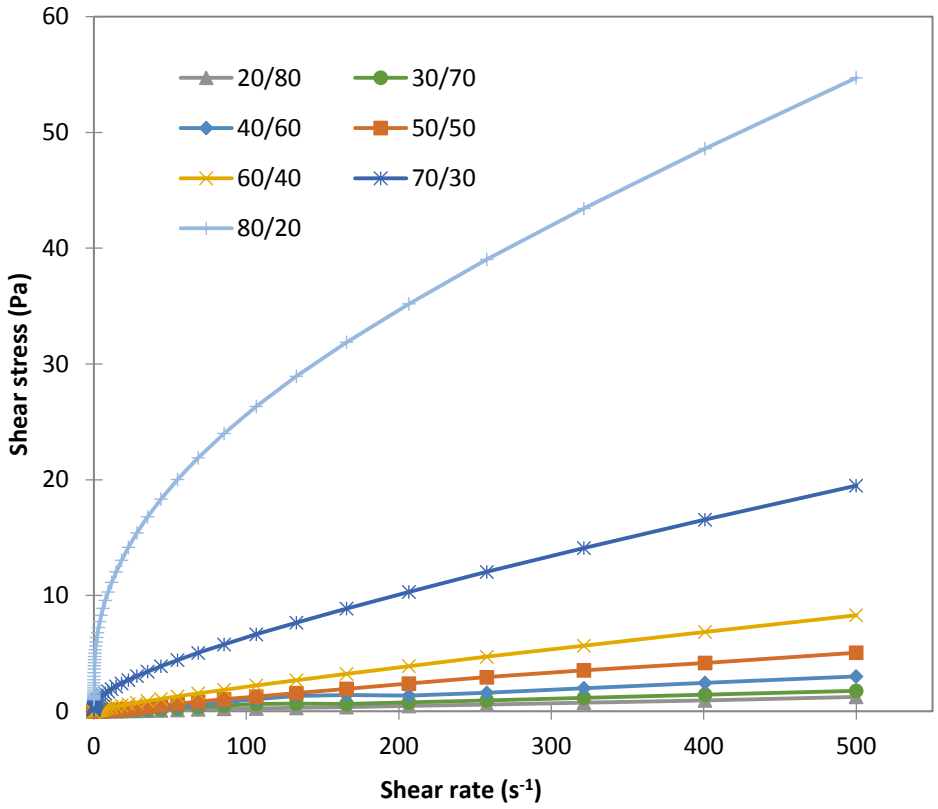


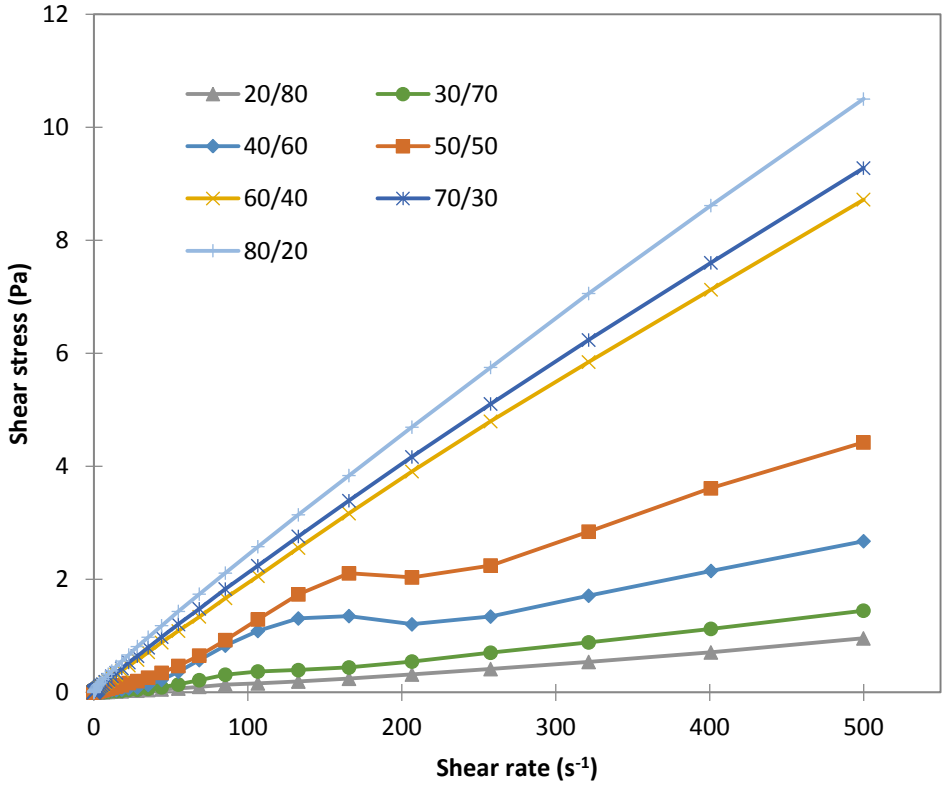
(B)

Figure 2

Figure(s) 3



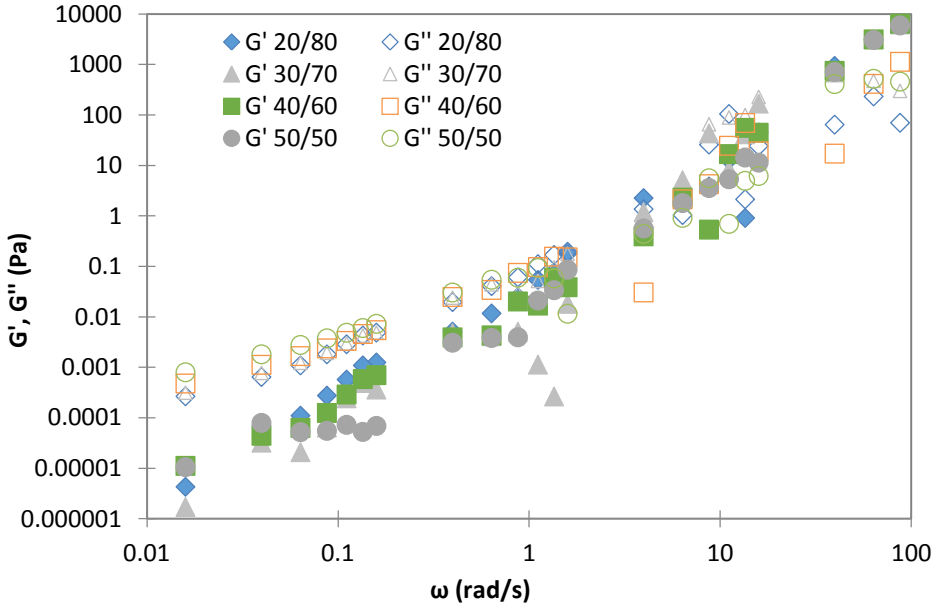
(A)



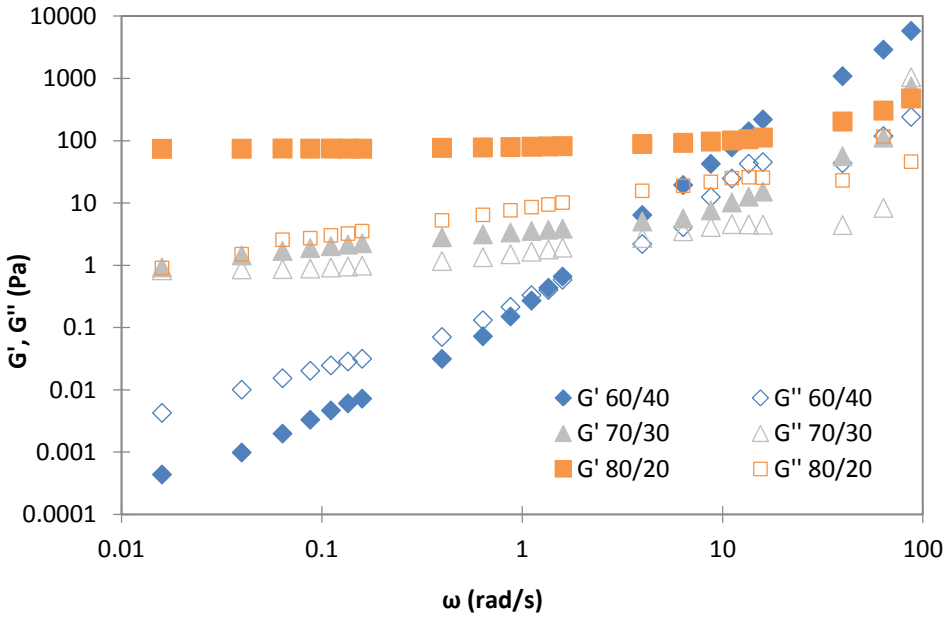
(B)

Figure 3

Figure(s) 4


10000
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
G', G'' (Pa)
0.01
0.1
1
10
100
ω (rad/s)
G' 20/80
G'' 20/80
G' 30/70
G'' 30/70
G' 40/60
G'' 40/60
G' 50/50
G'' 50/50

(A)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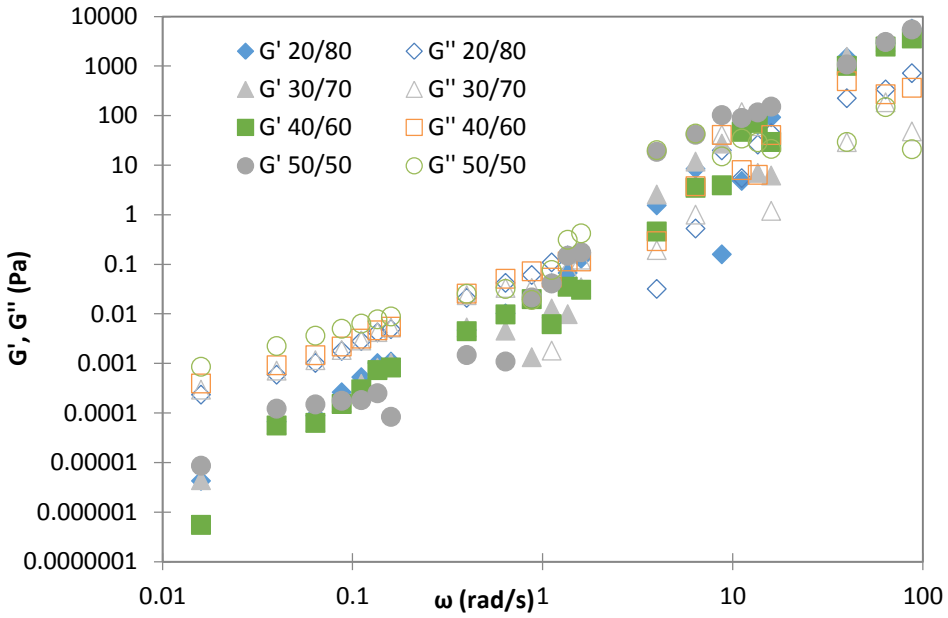
10
1
0.1
0.01
0.001
0.0001
G', G'' (Pa)
0.01
0.1
1
10
100
ω (rad/s)
G' 60/40
G'' 60/40
G' 70/30
G'' 70/30
G' 80/20
G'' 80/20

(B)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0.01
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0.0001
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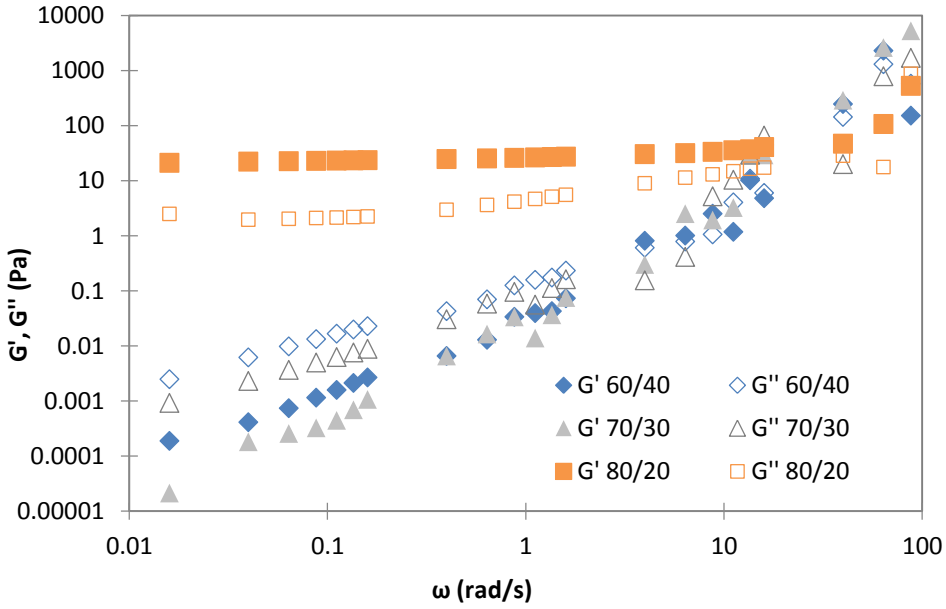
0.000001
0.0000001
G', G'' (Pa)
0.01
0.1
1
10
100
ω (rad/s)
G' 20/80
G'' 20/80
G' 30/70
G'' 30/70
G' 40/60
G'' 40/60
G' 50/50
G'' 50/50

(C)


10000
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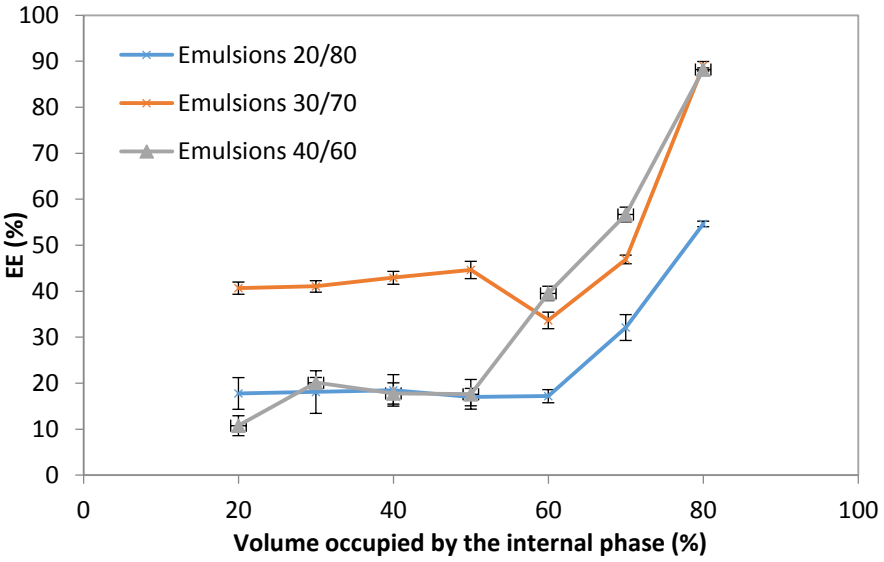
100
10
1
0.1
0.01
0.001
0.0001
0.00001
G', G'' (Pa)
0.01
0.1
1
10
100
ω (rad/s)
G' 60/40
G'' 60/40
G' 70/30
G'' 70/30
G' 80/20
G'' 80/20

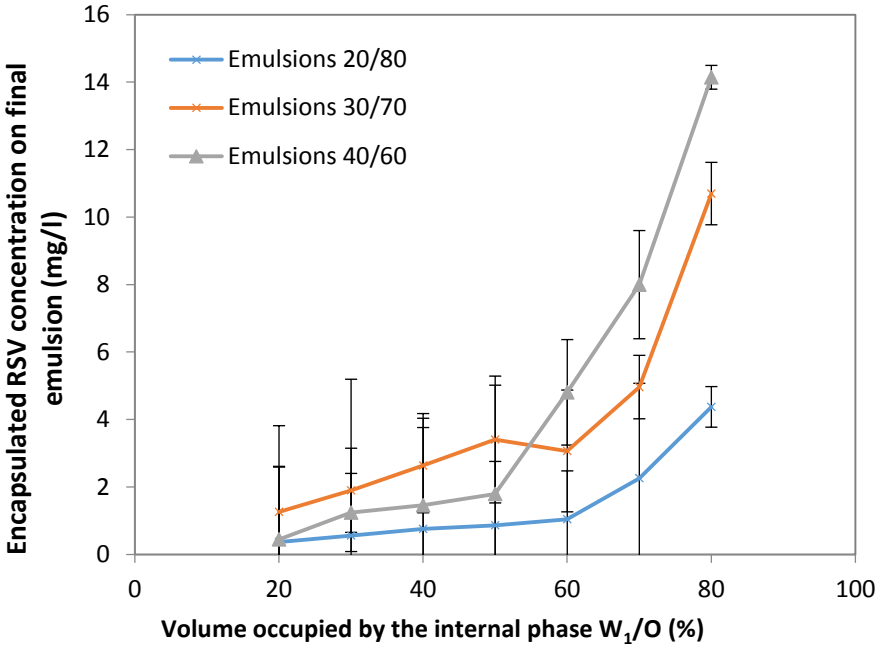
(D)

Figure 4

Figure(s) 5



(A)



(B)

Figure 5

Supplementary Material

Click here to download Supplementary Material: supplementary material_v3.docx

Credit author statement

Rocío DÍAZ-RUIZ: validation; formal analysis, resources, data curation, Writing - Original Draft

Lemuel MARTÍNEZ-REY: validation, resources, data curation

Amanda LACA: conceptualization, validation, Writing - Review & Editing

José Ramón ÁLVAREZ: conceptualization, validation, Writing - Review & Editing Writing - Review & Editing

Gemma GUTIÉRREZ: conceptualization, validation; Writing - Review & Editing; Supervision; Project administration

María MATOS: conceptualization, validation; Writing - Review & Editing; Supervision; Project administration