

Water in oil (w/o) and double (w/o/w) emulsions prepared with spans: microstructure, stability, and rheology

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Abstract The objective was to analyze the microstructure, stability, and rheology of model emulsions prepared with distilled water, refined sunflower oil, and different Spans (20, 40, 60, and 80) as emulsifiers. The effects of the water content and Span 60 concentration were studied. The lowest water contents led to w/o emulsions, whereas higher percentages gave w/o/w emulsions. Microscopy analysis showed that w/o/w emulsions of higher water contents had a lower number of internal water droplets. W/o emulsions were destabilized by coalescence and sedimentation, whereas creaming was observed in unstable w/o/w emulsions. In the last ones, the creaming stability decreased with increasing water content and enhanced with higher Span 60 concentration; the same effect was observed in their viscoelasticity: They were from unstable liquids to stable gels. Solid Spans (40 and 60) produced more consistent w/o/w emulsions at low water contents and more stable systems at high water percentages in comparison with liquid Spans (20 and 80).

Keywords Spans · W/o emulsion · W/o/w emulsion · Microstructure · Stability · Rheology

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Introduction

Spans are nonproteic emulsifiers with a higher hydrophobic than hydrophilic character (low HLB). They are sorbitan esters differentiated among themselves by the characteristics of their fatty acid chains. Span 40 (sorbitan monopalmitate) and Span 60 (sorbitan monostearate) are solid at room temperature because of their relatively long saturated hydrocarbon chains, the second one being longer; Span 20 (sorbitan monolaurate) and Span 80 (sorbitan monooleate) are liquid at room temperature as a result of the relatively short fatty acid chain of the first one and the long but unsaturated chain of the second one. The higher extent of chain–chain interactions in solid Spans favors the formation of more condensed monolayers at 22–42 °C, whereas liquid Spans remain as expanded type monolayers at the same temperature range [1]. With regard to the interfacial tension, previous studies showed that it was slightly lowered as the chain–chain interactions increased; nevertheless, there were not significant differences in tensioactivity between Spans at the critical micelle concentration [2]. Spans are used as emulsifying agents in cake mixes, baked goods, puddings, imitation whipped cream, and cosmetics, among others.

As w/o emulsions are less common than o/w ones, fewer studies are dedicated to them, although they are very important as butter and margarine are typical examples. As a general rule, it is accepted that emulsifiers should have more affinity to the continuous phase than to the dispersed one; thus, Spans are adequate to formulate this type of emulsions. Berg et al. [3] studied the microstructure of w/o emulsions prepared with other emulsifiers.

W/o/w emulsions are more complex systems, having two water phases and a lipid one. The first water phase is dispersed in oil, which in turn is dispersed in a continuous

second aqueous phase. The most common way to prepare this type of emulsion has two steps as follows [4]: (1) preparation of a w/o emulsion with a hydrophobic emulsifier by a homogenization method of high energy and (2) dispersion of this w/o emulsion in a second aqueous phase with a hydrophilic emulsifier but now with lower homogenization energy. Differences in homogenization energies are necessary to obtain very small water droplets inside oil droplets, whereas the second homogenization should produce large enough oil droplets to contain the first ones [5]. Hou and Papadopoulos [6] developed a technique where relatively large oil in water droplets are produced with micropipettes in which, in turn, smaller water droplets are injected. The w/o/w emulsions studied in the present work were obtained in one step by phase inversion during homogenization without the need of a hydrophilic emulsifier.

Stability is a very important parameter to know the applicability of an emulsion to produce more complex systems. Because the emulsions studied in this work are wanted to be used in the formulation of reduced lipid content foods, a relatively high stability is needed to get a product of invariable quality during the required time (transport, storage, etc.). The stability of different types of emulsions prepared with Spans was already analyzed by other authors [6–10], but there are not studies of w/o/w emulsions prepared in one step with Span as the only surfactant.

The objective of this work was to obtain w/o and w/o/w model emulsions with different Spans as a potential practical application to prepare reduced lipid content food systems. Characterization of emulsions were performed by microscopic analysis, droplet size distribution determination, light scattering measurements, and rheology studies.

Materials and methods

Materials

Refined sunflower oil (Molinos Río de la Plata S.A., Avellaneda, Argentina), distilled water, Span 20 (sorbitan monolaurate, Fluka Chemika 85544; Fluka Chemie, GmbH; Buchs, Switzerland; HLB=8.6; viscosity at 25 °C=4,250 cp), Span 40 (sorbitan monopalmitate, Sigma S-6885; Saint Louis; HLB=6.7; melting point=48 °C by differential scanning calorimetry), Span 60 (sorbitan monostearate, Sigma S-7010; Saint Louis; HLB=4.7; melting point=54 °C by differential scanning calorimetry), and Span 80 (sorbitan monooleate, Fluka Chemika 85548; Fluka Chemie, GmbH; Buchs, Switzerland; HLB=4.3; viscosity at 25 °C=1,000 cp).

Emulsion preparation

Different proportions by weight of oil and the corresponding Span were manually mixed. To allow homogenization of the mixture, solid Spans (40 and 60) were previously melted at 60 °C, whereas for liquid Spans (20 and 80), only a slight manual mixing at room temperature was enough. Proportions by weight of distilled water were added (total sample weight, 50 g), and mixtures were homogenized using an Ultraturrax T-25 (IKA-Works, Wilmington) with a S25N-8G rotor (IKA-Works; rotor/stator distance, 0.25 mm; rotor diameter, 6.1 mm) at 24,000 rpm during 2 min; in the cases of previous heating, homogenization was performed at 60 °C. In emulsions prepared with Span 60, the emulsifier concentration was varied from 0.5 to 5% w/w and water content from 10 to 90% w/w. In emulsions containing the other Spans, the emulsifier concentration was maintained at 1% w/w, and water content was varied from 10 to 70% w/w.

Light microscopy

Micrographs were obtained with a Leica DMLB optical microscope (Leica Microsystems, GmbH; Wetzlar, Germany) with an adapted digital camera (Leica DC100, Leica Microsystems, GmbH) operating at 200× magnification. To determine whether the continuous phase was water or oil, samples were stained with Ponceau red 4R hydrophilic dye (Dynamec, India), which gives a reddish color to the aqueous continuous phase. W/o/w emulsions were recognized when oil droplets were seen isolated and surrounded by a red continuous aqueous phase with little droplets (dispersed aqueous phase) inside of them.

Analysis of internal droplets

The number of internal water droplets per surface unit (μm^2) of oil in w/o/w emulsions was obtained by visual analysis of the micrographs. For each sample, ten oil droplets with a mean diameter above 3 μm were randomly selected. Visible internal water droplets of the selected oil droplets were counted. Oil-droplet diameters were measured with a micrometric ruler; mean values were calculated using two perpendicular diameters of each droplet because not all droplets were spherical. Analyzed total surface was calculated with mean diameters according to the two dimensions of the micrographs. Then, the ratio number of water droplets/ μm^2 of oil for each sample was calculated.

Droplet size distribution

Size distribution of oil droplets of w/o/w emulsions was obtained with a particle analyzer (Malvern Mastersizer

Micro Particle Analyzer, Malvern Instruments, Worcester-shire, UK). Sauter mean diameter (d_{32}), which is a measurement of the area formed during the homogenization process [11], was obtained from the droplet size distribution expressed as differential surface. Measurements were performed immediately after the emulsion preparation at least in duplicate.

Global stability

Global stability of emulsions was determined by light scattering measurements using a vertical scan analyzer (Quick Scan, Beckman Coulter, Fullerton). This equipment scans the sample along the tube where it is contained, giving a number of profiles of back scattering (%BS) and transmission percentages as a function of time and tube length [12]. Measurements were performed continually during 1 h and punctually after 1 day. From the obtained profiles, mean values of %BS were calculated as follows: for w/o emulsions in the 20- to 50-mm zone (%BS_{20–50}) and for w/o/w ones in the 6.5- to 10-mm zone (%BS_{6.5–10}), corresponding to the medium and bottom parts of the tube, respectively. As a method to determine the kinetics of destabilization, for w/o emulsions, it was defined as the coalescence percentage (%C) as $[(BS_{in\ 20-50} - BS_{f\ 20-50}) / BS_{in\ 20-50}] \times 100$, where %BS_{in 20–50} is the initial %BS_{20–50} value, and %BS_{f 20–50} is the %BS_{20–50} value at the required time. For w/o/w emulsions, it was defined as the creaming constant ($K_{0,1}$) as $(BS_{in\ 6.5-10} \times t_{0,1})^{-1}$, where $t_{0,1}$ is the time when %BS_{6.5–10} diminished 10% of its initial value (%BS_{in 6.5–10}). When emulsions keep stable after 24 h, the $K_{0,1}$ value would be lower than 10^{-4} min^{-1} ; thus, $K_{0,1}$ was considered null in those cases.

Rheology

Rheological analysis of w/o/w emulsions was performed by a Haake RS600 rheometer (Haake, Karlsruhe, Germany) with a serrated PP35-S rotor using a 1-mm gap parallel-plate sensor. Temperature was maintained at 20 °C. Experimental data were obtained by recording the elastic modulus (G'), viscous modulus (G''), and $\tan \delta$ (G''/G') as a function of oscillation frequency (0.01–10 Hz range) within the linear viscoelasticity range (stress=3 Pa). Tests were conducted at least in duplicate.

Results and discussion

Microstructure

Light microscopy showed that Spans 40 and 60, solid emulsifiers at room temperature, led to w/o emulsions of up

to 20% water and to w/o/w emulsions at higher water contents. The last result is attributed to phase inversion (from w/o to w/o/w) during homogenization, as Spans would initially form a temporary w/o emulsion because of their higher affinity to the lipid phase than to the aqueous one. A similar effect was detected with Spans 20 and 80, liquid at room temperature; however, in these cases, the limit of phase inversion was observed at lower water contents (between 10 and 15% water; same results are observed if the preparation temperature is 60 °C). These results indicate that with these emulsifiers and the employed homogenization method, w/o emulsions with water content higher than 20% cannot be formed. However, this allows to prepare w/o/w emulsions in one step without the need of a second homogenization and a hydrophilic emulsifier.

The effect of the water content (from 20 to 70%) on the microstructure of emulsions prepared with Span 60 (1%) can be observed in Fig. 1. The change from a w/o to a w/o/w emulsion because of the increment of water content (from 20 to 30%) can be appreciated in Fig. 1a (w/o) and Fig. 1b (w/o/w). Water droplets corresponding to the internal phase of w/o/w emulsions had diameters up to 1 μm . A visual analysis of the micrographs indicated that the number of internal water droplets per oil surface decreased with increasing water content within the range of 30–50% water (Table 1). Although this seemed to be a paradoxical result, it can be explained by the fact that emulsions with less water content are closer to the w/o type, where all the water is dispersed in the oil phase. During homogenization, a lower water content would allow to produce more water droplets because of a more gradual shift from w/o to w/o/w; this would explain the higher amount of internal water droplets in w/o/w emulsions with relatively low water contents (Fig. 1b and c). At contrary, within 50–70% water, the number of water droplets/ μm^2 oil ratio increased with increasing water content. However, the oil droplet size (and analyzed oil surface) decreased in a higher extent than the number of internal water droplets per oil droplet, which explains the consequent increase of the ratio (Table 1). It was also observed that oil droplets had lower packing as water content increased because of the higher proportion of continuous water phase. This fact would be an important factor for the emulsion consistency, which decreased with increasing water content.

To analyze differences between Spans, micrographs of w/o/w emulsions prepared with Span 20 (Fig. 2a), Span 40 (Fig. 2b), Span 60 (Fig. 2c), and Span 80 (Fig. 2d) were compared. At the same water content (50%) and emulsifier concentration (1%), Spans 40 and 60 seemed to give similar w/o/w emulsions; Span 80 showed a smaller size and higher number of internal water droplets, whereas Span 20 gave the lowest number of them. Thus, the number of water

droplets would depend on the hydrophilic-lipophilic balance (HLB) of the emulsifiers, as Span 20 is the most hydrophilic of the four assayed Spans, and Span 80 has the most hydrophobic character. This result could be explained by a slower phase inversion during homogenization as the hydrophobicity of the emulsifier increases, allowing the formation of more water droplets.

Figure 3 shows the effect of Span 60 concentration and water content on the oil droplet size of w/o/w emulsions. The oil droplet surface distributions were bimodal (Fig. 3a and d). An increase of Span concentration led to smaller droplet sizes because of the possibility of forming more interfacial area (Fig. 3c). Sauter mean droplet diameter

(d_{32}), obtained from a bimodal surface distribution, is determined by both the area under each peak and the modes (droplet sizes at maximum points of peaks). As Span concentration increased, the mode value of the peak at smaller droplet sizes (mode 1) did not follow a pattern, whereas the mode value of the peak at larger droplet sizes (mode 2) had a constant decrease (Fig. 3b), showing a direct relationship with the variation of the d_{32} value (Fig. 3c). This last result indicates that the d_{32} value is affected mostly by the larger droplet sizes.

Figure 3d shows that the increasing water content led to a decrease of the surface percentages of modes and a parallel increase of the percentage of intermediate droplet

Fig. 1 Micrographs (200 \times). Effect of water content on emulsions with Span 60 (1%): **a** 20% water; **b** 30% water; **c** 40% water; **d** 50% water; **e** 60% water; and **f** 70% water

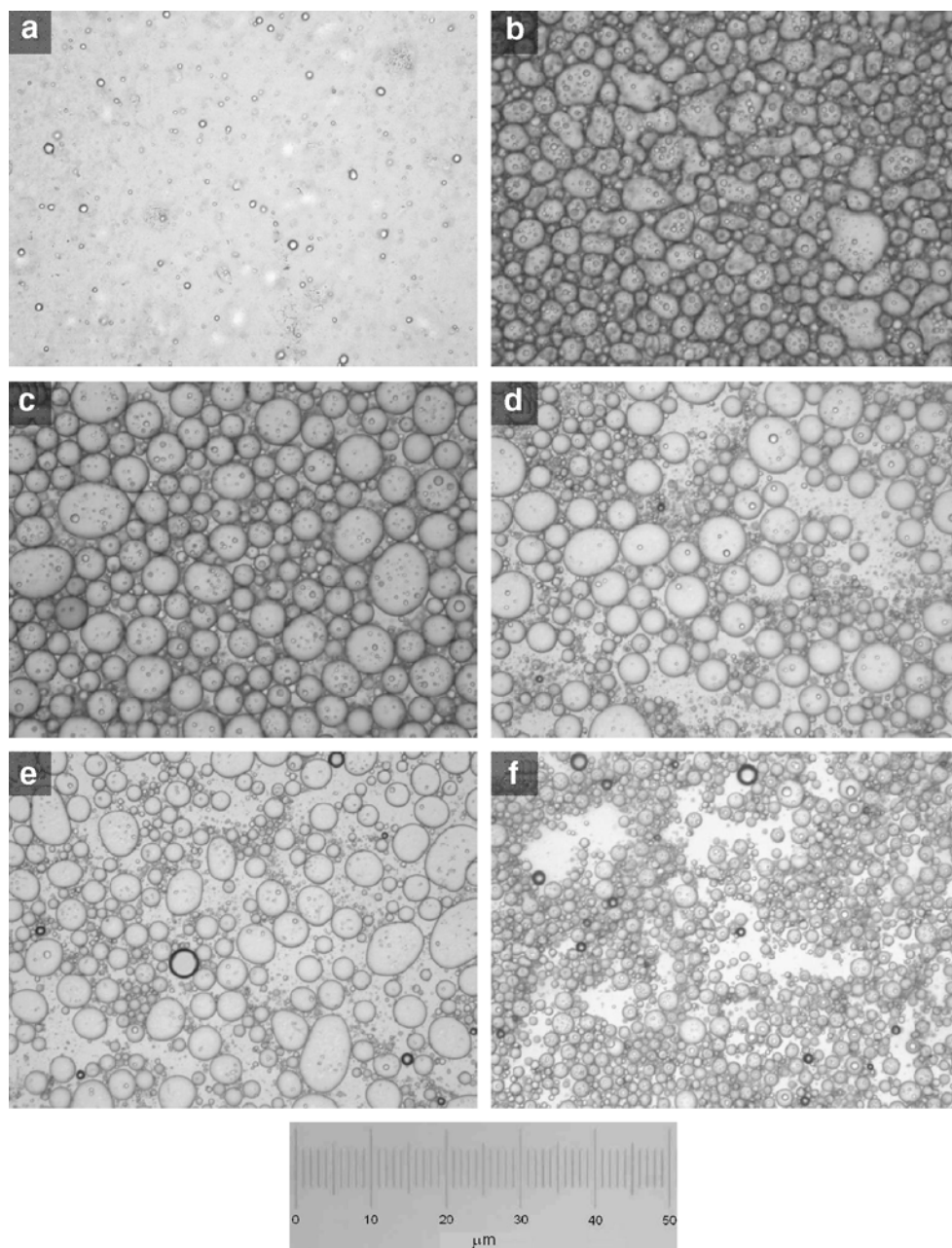


Table 1 Effect of water concentration on the number of visible internal droplets of water per oil surface in w/o/w emulsions with 1% Span 60

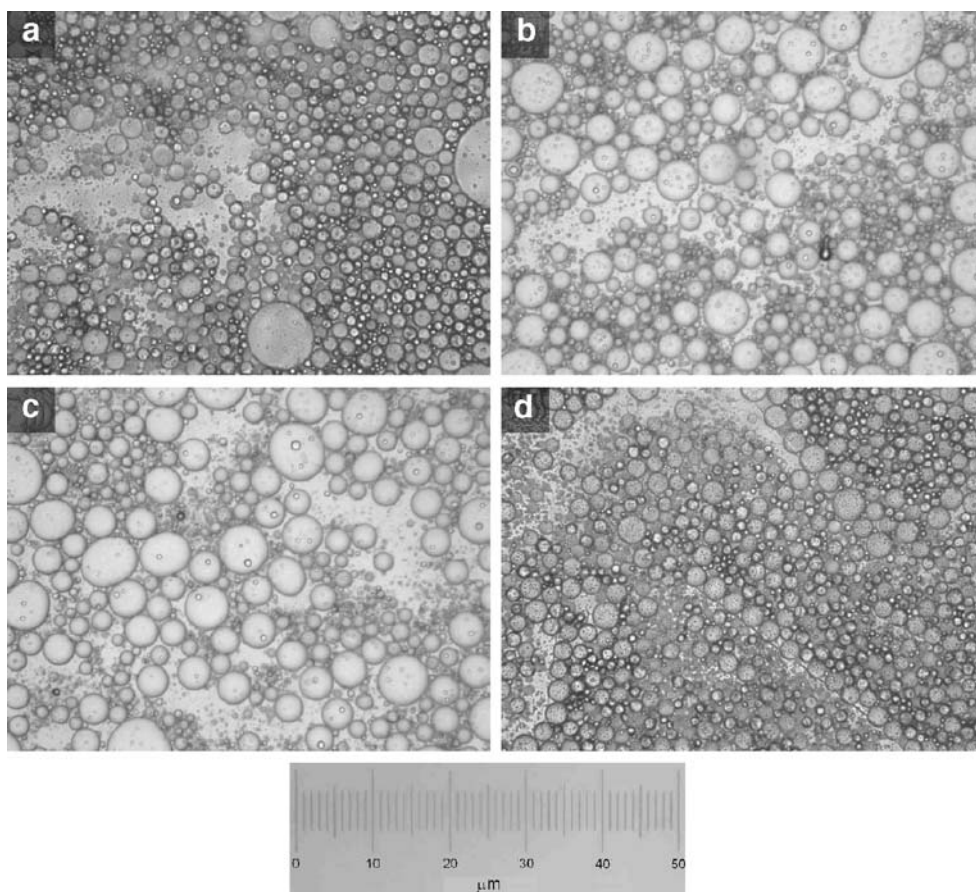
| Water percentage (w/w) | Analyzed oil surface (μm^2)* | Number of visible water droplets* | Number of water droplets/ μm^2 of oil |
|------------------------|---|-----------------------------------|--|
| 30 | 147.1 | 119 | 0.809 |
| 40 | 291.2 | 129 | 0.443 |
| 50 | 251.5 | 73 | 0.290 |
| 60 | 182.8 | 58 | 0.317 |
| 70 | 84.2 | 45 | 0.534 |

*Data corresponding to ten oil droplets

sizes; these results agreed with those previously observed in o/w emulsions [13]. Again, the variation of mode 1 was aleatory, and mode 2 varied the same way as the d_{32} values (Fig. 3e and f). A slight increase in particle size was detected with increasing water content within 30–50% water (Fig. 3f) probably because of a higher packing given by the lower volume of aqueous phase, which would prevent mobility and coalescence of droplets during homogenization. However, from 50% water, the droplet size had a marked decrease with increasing water content; at 50–90% water, particles would have reached and kept an almost constant mobility degree. Thus, in these cases, the main factor would be that a lower oil content favors the formation of smaller particle sizes as it was previously determined for o/w emulsions [13].

With regard to the influence of Span type, in w/o/w emulsions containing 1% Span and 50% water, a slight increase of d_{32} was observed with a decreasing HLB of the emulsifier: Span 20, $6.03 \mu\text{m}$ (± 0.04) < Span 40, $6.45 \mu\text{m}$ (± 0.08) < Span 60, $6.68 \mu\text{m}$ (± 0.05) \approx Span 80, $6.69 \mu\text{m}$ (± 0.01). These values would indicate that, at least to some extent, the higher emulsifier affinity to the continuous aqueous phase, the smaller the size of oil particles.

The relative mean size of water droplets of w/o emulsions with the same water content can be estimated by the initial %BS_{20–50}; the correlation between mean droplet size and %BS was previously demonstrated by Palazolo et al. [14]. Initial %BS_{20–50} values of w/o emulsions were ordered this way: Span 60 < Span 40 < Span 80 < Span 20 (Table 2, time=0). These results indicate that

Fig. 2 Micrographs (200 \times). Effect of Span type on w/o/w emulsions with 1% emulsifier and 50% water: **a** Span 20; **b** Span 40; **c** Span 60; and **d** Span 80

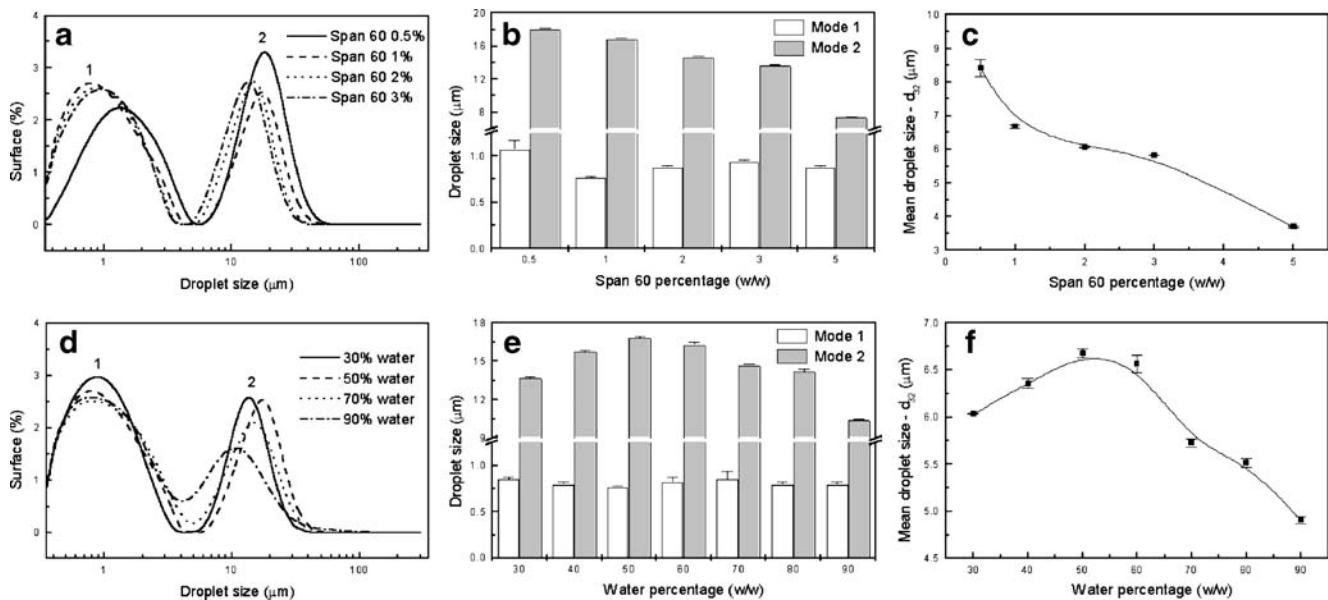


Fig. 3 Effect of Span 60 concentration on w/o/w emulsions with 50% water on: **a** surface droplet size distribution; **b** modes (droplet sizes at maximum points of peaks); and **c** mean droplet size (d_{32}). Effect of

water content on w/o/w emulsions with Span 60 (1%) on: **d** surface droplet size distribution; **e** modes; and **f** mean droplet size (d_{32})

liquid Spans produce w/o emulsions with smaller initial size of water droplets than solid Spans. In all cases, Span percentage was more than the critical micelle concentration; at these conditions, differences in interfacial tension values between Spans were not significant [2]; thus, the differences in droplet sizes would not be attributed to the different tensioactivity. The size of the droplets produced by a homogenizer depends on a balance between the following two opposing physical processes: droplet disruption and droplet coalescence. Emulsions are highly dynamic systems in which the droplets continuously move around and frequently collide with each other. During homogenization, droplet–droplet collisions are particularly rapid because of the intense mechanical agitation of the emulsion, and the resistance of an interfacial film to coalescence depends on the emulsifier concentration, as well as their structural and physicochemical properties [15]. When the interface is composed of solid Spans, higher collision efficiency would occur because of more interactive hydrocarbon chains exposed toward the continuous lipid phase. Liquid Spans have less interactions between fatty acid chains in comparison with solid Spans [1] because of the shorter chain of Span 20 and the unsaturated chain of Span

80; this would explain the smaller droplet sizes formed with liquid Spans. Span 60 has a longer saturated hydrocarbon chain than Span 40, which would explain the slightly smaller water droplets produced by the second one. Then, the capability of Spans to produce w/o emulsions with more interfacial area would depend mainly on the chain–chain interactions between the interfaces during homogenization.

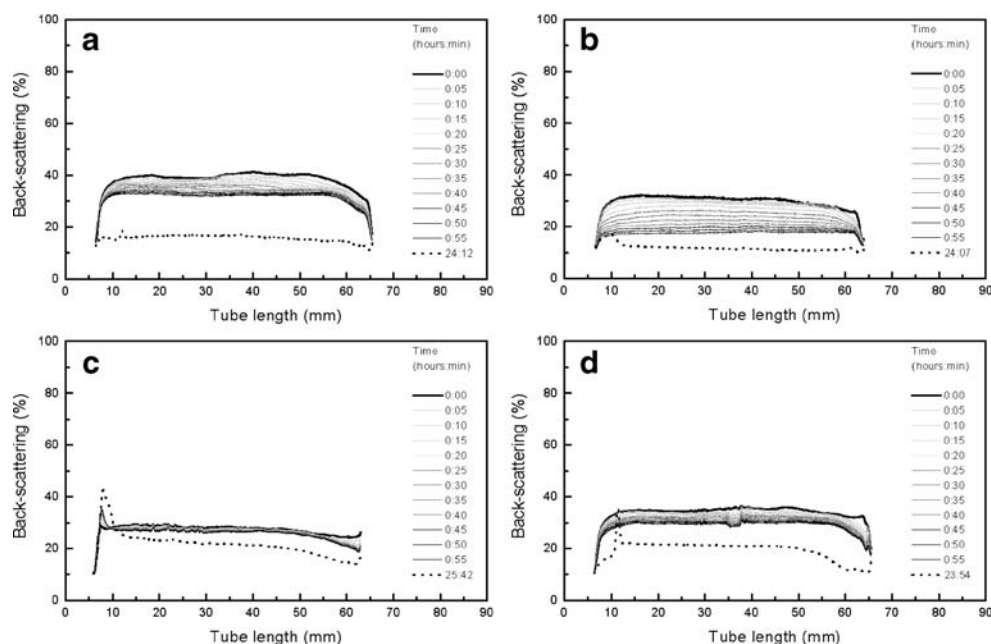
Stability

The stability study showed that w/o emulsions were destabilized by coalescence and sedimentation processes, as it can be observed in the %BS profiles (Fig. 4). Coalescence of water droplets is evidenced by the %BS diminution along the tube. The %C values after 1 h were ordered this way: Span 60 < Span 80 < Span 20 < Span 40 (Table 2, time=1 h). According to the %C values, Span 80 gave a slightly more stable w/o emulsion against coalescence than Span 20 after 1 h, whereas the emulsion prepared with Span 40 was clearly the most unstable. Span 60 would seem to give the most stable w/o emulsion against coalescence, although it must be considered that it gave a relatively low initial %BS_{20–50} value to be diminished. In addition, the emulsion prepared

Table 2 Variation of back-scattering (%BS_{20–50}) and coalescence percentage (%C) with time after preparation in w/o emulsions with 1% Span and 10% water

| Span | Time=0 | | Time=1 h | | Time=24 h | |
|------|----------------------|------|----------------------|------|----------------------|------|
| | %BS _{20–50} | %C | %BS _{20–50} | %C | %BS _{20–50} | %C |
| 20 | 39.9 | 20.1 | 31.8 | 16.4 | 16.4 | 58.9 |
| 40 | 30.9 | 44.5 | 17.1 | 11.5 | 11.5 | 62.9 |
| 60 | 27.9 | 5.6 | 26.4 | 21.6 | 21.6 | 22.8 |
| 80 | 35.1 | 16.7 | 29.3 | 21.1 | 21.1 | 40.0 |

Fig. 4 Profiles of back scattering (%BS) as a function of time and tube length for w/o emulsions: **a** Span 20 (1%) with 10% water; **b** Span 40 (1%) with 10% water; **c** Span 60 (1%) with 10% water; and **d** Span 80 (1%) with 10% water



with Span 60 showed fast accumulation of particles at the bottom of the tube (sedimentation) as it is evidenced by the increase of %BS at that zone during the first hour (Fig. 4c), which is not seen with the other emulsifiers during that time; this may be attributed to the larger initial size of water droplets given by this emulsifier.

After 24 h, although the order of stability was kept, the difference between the %C values of Spans 80 and 20 increased strongly, whereas the difference between Spans 20 and 40 became relatively small (Table 2, time=24 h). With regard to Span 60, its %C value was quite lower than Span 80, but they both reached to a similar %BS_{20–50} value after 24 h, higher than the other Spans. These last results could be attributed to simultaneous coalescence and sedimentation processes, as a visual analysis of the samples showed formation of an aqueous phase at the bottom of the tubes after 24 h (confirmed by staining with Ponceau red hydrophilic dye). Although Span 60 showed rapid sedimentation at the beginning, the higher %BS_{20–50} after 24 h would indicate that a remnant of water droplets stayed dispersed in the oil phase during more time with this emulsifier as well as with Span 80 in comparison with the other surfactants; this may be confirmed by the gradient of

%BS (at 24 h) observed with Spans 60 (Fig. 4c) and 80 (Fig. 4d), which decreases from the bottom to the top of the tube, showing the process of sedimentation that had already been concluded with Spans 20 (Fig. 4a) and 40 (Fig. 4b). Spans 60 and 80 have lower HLB values than the other two surfactants, so a higher affinity of the emulsifier to the continuous lipid phase would allow to produce more stable and dispersed water droplets.

A microscopic analysis of the aqueous phase formed after 24 h indicated the presence of oil droplets, which explains null or very low transmission values (data not shown). The formation of those oil droplets could be attributed to phase inversion of a high water-concentrated w/o emulsion because of the accumulation of water droplets at the bottom of the tube after sedimentation. The number and size of these new droplets would also be determined by the HLB of the emulsifiers, as at the bottom of the tube and after 24 h, emulsions prepared with Spans 60 (Fig. 4c) and 80 (Fig. 4d) showed higher %BS values (over the initial % BS value), which indicates the existence of more and/or smaller oil droplets. In the case of Span 80, it seems that the oil droplets ascended to the top of the aqueous phase because of gravitational separation or creaming.

Fig. 5 Profiles of back scattering (%BS) as a function of time and tube length for w/o/w emulsions: **a** Span 60 (0.5%) with 50% water (unstable) and **b** Span 60 (2%) with 50% water (stable)

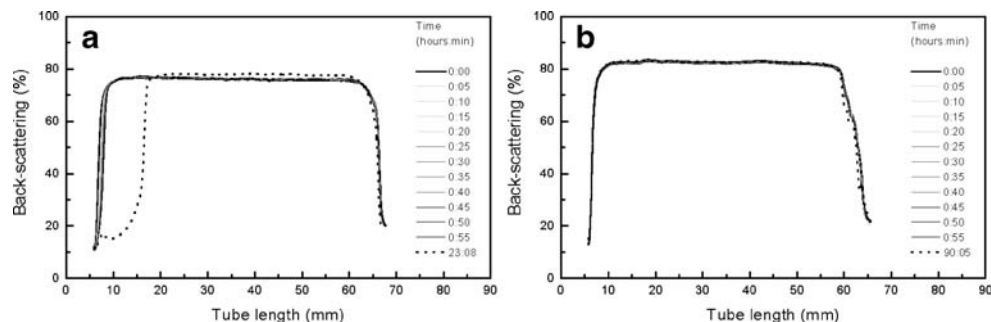


Fig. 6 **a** Effect of water and Span 60 concentrations on the creaming constant ($K_{0,1}$) for w/o/w emulsions. **b** Effect of water concentration and Span type on $K_{0,1}$ for w/o/w emulsions

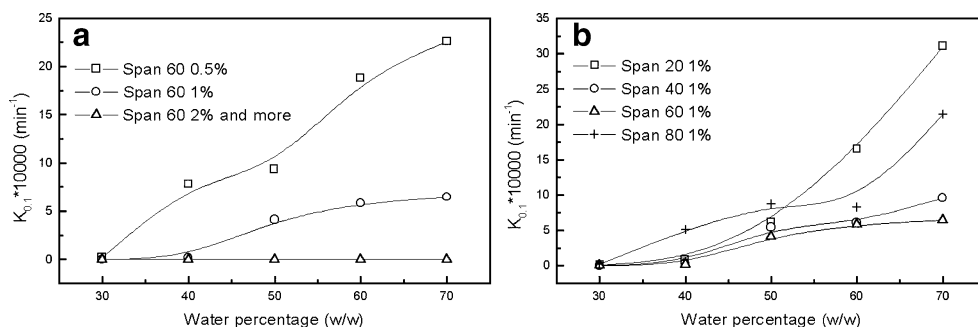


Figure 5 shows, as examples, different %BS profiles corresponding to two w/o/w emulsions, one unstable (Fig. 5a) and other stable (Fig. 5b). Creaming was observed as the destabilization process in those w/o/w emulsions that were unstable. This was evidenced by the displacement of the %BS profiles from the bottom toward the top of the tube (from left to right in the graphic), indicating the ascent of oil droplets because of gravitational separation. Coalescence was not observed in these emulsions; this indicates that with every Span, the interfacial film had enough rigidity to avoid its rupture when two droplets collide; although with solid Spans, the film would be more condensed as a result of higher chain–chain interactions [1]. The formation of micelles and their eventual migration from the dispersed oil phase to the interface would explain the relatively high rigidity of the films. The formation of a hydration layer around the oil droplets, which would be induced by the exposition of the polar heads of the emulsifiers, could also contribute to coalescence stability.

Figure 6 summarizes the analysis of the stability of w/o/w emulsions, expressed as the creaming constant $K_{0,1}$. As it was expected, it was observed that at same water content, the stability increases ($K_{0,1}$ decreases) with increasing emulsifier concentration (Fig. 6a); this is attributed to the smaller oil droplet size given by the increment of the Span (Fig. 3c), as smaller particles have less tendency to suffer a creaming process. From 2% Span 60, $K_{0,1}=0$ in the water concentration range that was analyzed; all these samples were totally stable during at least 4 days (some of them lasted several weeks). With regard to the influence of water percentage, at relatively low Span concentrations (0.5–1%), an increase of water content produced a decrease of the stability of these emulsions ($K_{0,1}$ increases) probably because of the higher mobility of oil droplets given by the increase of the continuous aqueous phase; this favors the displacement of the droplets from the bottom to the upper part of the tube.

The study of the effect of the Span type on the stability can be observed in Fig. 6b. At 30% water, creaming stability was very high with every emulsifier ($K_{0,1} \approx 0$) because of the relatively high packing degree of oil droplets. At higher water percentages, emulsions prepared

with Span 60 were the most stable ones, as it is evidenced by the always lower $K_{0,1}$ values; Span 40 gave slightly less stable emulsions than Span 60, whereas Spans 20 and 80 produced the least stable ones. These results indicate that the solid Spans are capable to form more stable emulsions than the liquid ones. In this case, there is not a direct relationship between stability and droplet size. The higher stability given by solid Spans would be attributed to the formation of a larger hydration layer around the oil droplets. This larger hydration could be induced by a better exposition of polar heads toward the continuous aqueous phase because of the higher chain–chain interactions between Span molecules in the interfacial film. The slight differences between Spans 40 and 60 could be explained by the longer saturated hydrocarbon chain of the second one, which favors the chain–chain interactions. No appreciable changes in stability were observed when emulsions with liquid Spans were prepared at 60 °C; it was previously demonstrated that the films formed with liquid Spans remain as expanded type at 22–42 °C [1]; thus, in these cases, cooling would not have a significant effect on the microstructure of the emulsion.

Rheology

Graphics of G' , G'' , and $\tan \delta$ are presented in Fig. 7, showing the effects of Span 60 concentration, water content, and Span type on the rheological behavior of some of the most stable w/o/w emulsions. An increase of Span 60 concentration (at the same water content, 50%) led to higher G' and G'' values (Fig. 7a and b), producing more viscoelastic and consistent emulsions. As it was previously observed, higher Span concentration led to smaller oil droplet sizes (Fig. 3c); this would favor the interaction of oil droplets because of the higher number of particles surrounded by a hydration layer, leading to the formation of gel-like structures. This result is also evidenced by the lower $\tan \delta$ values given by a higher content of the emulsifier (Fig. 7c), confirming the tendency to form more elastic systems.

In the case of the effect of water percentage (at same Span 60 concentration, 1%), G' and G'' increased and $\tan \delta$

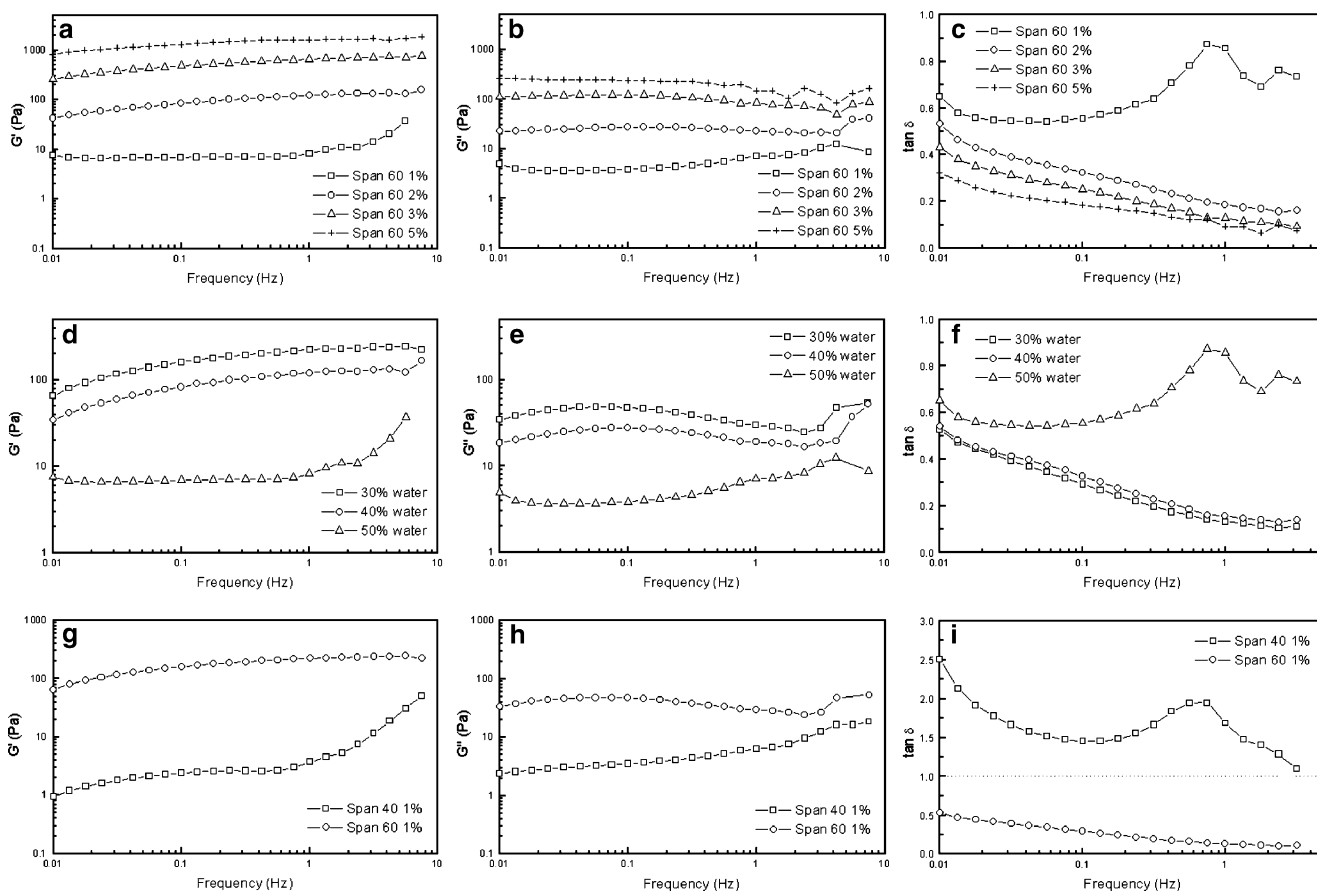


Fig. 7 Rheology studies for w/o/w emulsions. Effect of Span 60 concentration (water content, 50%) on **a** G' (elastic modulus); **b** G'' (viscous modulus); and **c** $\tan \delta$ (G''/G'). Effect of water content (Span

60 concentration, 1%) on **d** G' ; **e** G'' ; and **f** $\tan \delta$. Effect of Span type (water content, 30%) on **g** G' ; **h** G'' ; and **i** $\tan \delta$

decreased with decreasing water content (Fig. 7d,e, and f, respectively); these results are attributed to the higher packing of oil droplets because of the lower proportion of continuous water phase, as it was previously observed by microscopy (Fig. 1). Within 30–50% water, the emulsions were more elastic than viscous systems ($\tan \delta < 1$; Fig. 7f); at higher water contents, the emulsions were liquid, and the applied stress was out of their linear viscoelasticity range. These results indicate that the effects of Span 60 concentration and water content on the rheology of these emulsions are directly related to their stability (Fig. 6a).

W/o/w emulsions prepared with Spans 20 and 80 were liquid even at low water contents (30%); therefore, a rheological analysis of them was not carried out. Spans 40 and 60 gave clearly more consistent systems, and their rheological behaviors were compared. Figure 7g and h shows that Span 60 produced more viscoelastic emulsions than Span 40 at the same water content (30%) and emulsifier concentration (1%); moreover, the $\tan \delta$ value for Span 40 was over the line of $\tan \delta = 1$, indicating more viscous than elastic characteristics ($G'' > G'$; Fig. 7i). These differences in viscoelasticity at 30% water did not mean a different stability because every Span gave a stable

emulsion at that water content (Fig. 6b). The larger hydration layer formed as a consequence of a more condensed film would explain the different consistencies of highly packed emulsions. Then, at 30% water, the stability would be mainly given by the packing of oil droplets, whereas the characteristics of the interfacial film would govern the viscoelasticity of the emulsion. On the other hand, at 60–70% water, all Spans gave liquid emulsions. These results would confirm that the differences in creaming stability at high water contents are due to the formation of larger hydration layers with solid Spans, as the oil droplets are more dispersed than packed in the continuous aqueous phase.

Conclusions

W/o emulsions were formed with percentages of water between 10 and 20%, whereas at higher percentages, w/o/w emulsions were obtained. For the last ones, it was determined that the higher the water content, the lower the amount of internal water droplets and packing degree of oil droplets.

Sizes of oil droplets in w/o/w emulsions decreased with increasing Span 60 concentration; water content had an influence on droplet sizes in a lower extent. The magnitude of the interactions between fatty acid chains of Spans seemed to determine the water droplet size of w/o emulsions, whereas the HLB of the emulsifiers was a more important factor of stability. The destabilization processes of w/o emulsions were coalescence and sedimentation, whereas creaming was observed in w/o/w emulsions. Both stability and viscoelasticity of w/o/w emulsions increased with the increasing Span 60 concentration and/or decreasing water content. Larger hydration layers in w/o/w emulsions prepared with solid Spans would explain both their higher consistency at low water contents and their higher stability at high water percentages, in comparison with liquid Spans. This type of emulsions could be promising to produce creams and mayonnaise with low lipid content, with the possibility of isolating, by inclusion in the internal aqueous phase, incompatible substances with those in the continuous aqueous phase.

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