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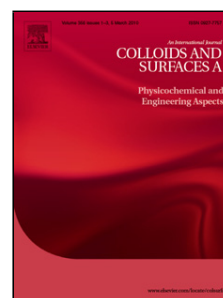
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RHEOLOGY, MICROSTRUCTURAL CHARACTERIZATION AND PHYSICAL STABILITY OF W/ α -PINENE/W EMULSIONS FORMULATED WITH COPOLYMERS

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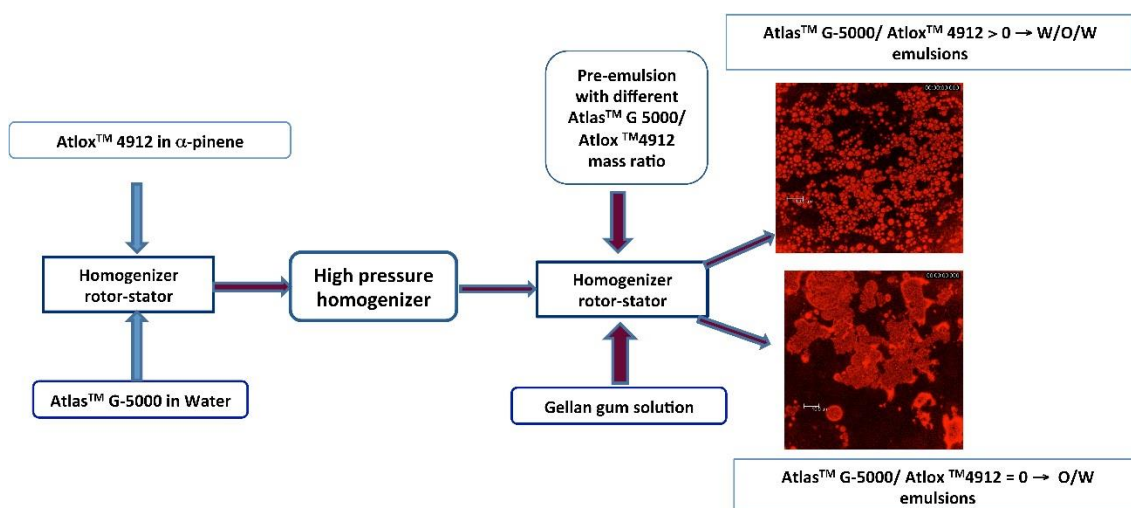
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Graphical abstract



Abstract

In this work the microstructure and the physical stability of emulsions containing α -pinene and gellan gum were studied as a function of Atlas™ G-5000/Atlox™ 4912 copolymer mass ratio, R , used as emulsifier. In order to reach this objective, electrolytic conductivity measurements, laser diffraction, confocal laser scanning microscopy (CLSM) and rheology were used. Electrolytic conductivity tests along with CLSM supported the formation of W/ α -pinene/W multiple emulsions in all systems containing the most hydrophilic copolymer. Parameter R did not significantly influence either the mean diameters or the span when both copolymers were used. However the lack of Atlas G-5000, not only did it provoke an important increase of mean diameter of droplets, but it also caused the formation of a simple O/W emulsion instead of a multiple emulsion. All emulsions showed shear thinning flow behaviour. The

mechanical spectra of multiple emulsions with $R \geq 2$ were similar to each other and independent of aging time. A reduction of R led to a decrease in G' and G'' values and aging time yielded increasing G' values probably due to the occurrence of incipient creaming. Conversely, the emulsion without AtlasTM G-5000 showed a drop of viscoelastic moduli with aging time due to a coalescence process. These multiple emulsions may find applications in agrochemistry since some active ingredients may be encapsulated in the inner phase, enabling the delivery of hazardous ingredients in a safer way, along with the use of α -pinene which is an easily biodegradable green solvent.

Keywords: multiple emulsion, physical stability, green solvent, amphiphilic block copolymer, high pressure homogenizer.

1. Introduction

Multiple emulsions can be defined as “emulsions of emulsions”, which consist of dispersed droplets (globules) that contain smaller droplets of different phases. Frequently, they are classified depending on the number (1) or on the nature (2) of internal droplets present in the emulsion globules: 1) into three groups [1, 2]: a) type A, where the multiple emulsion globule contain one large internal droplet, b) type B, where there are several small internal droplets in the globules of multiple emulsion and c) type C, where the globules of the multiple emulsion contain a large number of internal droplets 2) into two groups [3]: water/in oil/in water (W/O/W), and oil/in water/in oil (O/W/O).

The potential applications of multiple emulsions are numerous, especially in areas such as drug delivery systems, cosmetics [4, 5], foods [6] or agrochemicals [7, 8]. In the latter field, the W/O/W multiple emulsions are a good option to satisfy the trend to develop new formulations of pesticides, which must be cleaner and safer for users. This fact is due to the possibility to encapsulate active materials into the inner drops, which can be released under certain environmental conditions.

Recently, in order to develop more ecofriendly and safer formulations, the replacement of traditional organic solvents (oil phase) by biodegradable solvents is increasingly promoted. In order to accomplish this goal, several authors have studied the applications of solvents obtained from renewable sources such as essential oils, which may be used as organic phase in simple and multiple emulsions [8-13]. An interesting essential oil for the formulation of agrochemical emulsions is α -pinene, a terpenic solvent usually

obtained from the pine bark or by distillation, which provides some activity as bio-based pesticide [9, 10, 14].

Besides the typical destabilization mechanisms affecting simple emulsions, there are further mechanisms of instability in W/O/W multiple emulsions. Multiple drops may coalesce with other oil droplets (simple or multiple) or they could lose their internal droplets. The occurrence of an osmotic gradient leads to either swelling or shrinkage of the internal droplets depending on the direction of this gradient. Another possible breakdown mechanism may be coalescence of the internal aqueous droplets within the oil phase. A combination of these mechanisms may frequently take place such that the actual breakdown mechanisms remain usually unclear [3]. There is another type of destabilization mechanism which takes place by the coalescence of internal drops with the external phase. The appearance of this mechanism usually is caused by the presence of a hydrophilic surfactant in the outer aqueous phase [15, 16, 17]. In fact, this destabilization mechanism is responsible for encapsulation efficiency values lower than 1. The encapsulation efficiency is the ratio of water actually encapsulated in the multiple emulsion to the water content of the primary W/O emulsion [18].

However a major problem associated with W/O/W emulsions is creaming, which is probably due to the large size of the multiple drops. Creaming may be reduced by using a thickening agent in the external aqueous phase. In this work a commercial biopolymer obtained by fermentation, gellan gum, has been used.

In order to obtain stable multiple emulsions, two surfactants are needed, one of them of low HLB, to stabilize the w/o interface (lipophilic) and the other of high HLB, to stabilize the o/w interface (hydrophilic). This paper will explore the use of a combination of polymeric surfactants as emulsifiers. Namely, we report the performance as emulsifiers of an AB hydrophilic nonionic polyalkylene oxide (EO-PO) block copolymer, AtlasTM G-5000 (HLB: 16.9), and ABA hydrophobic block copolymer, AtloxTM 4912 (HLB: 5-6), obtained by esterification of poly (12-hydroxystearic acid), PHSA (A) with poly alkylene glycols (B). In a previous work, this combination of polymeric surfactants was used to prepare in a single step multiple emulsions containing 2-ethylhexyl lactate as dispersed phase [17, 18].

W/O/W double emulsions can be prepared via a phase inversion method [19], one-stage emulsification method [17], or two-stage emulsification method. Firstly, the inner W/O emulsion is obtained by traditional shear-rate homogenizer devices. Secondly, a

multiple emulsion is produced dispersing this W/O emulsion in an aqueous solution [3, 20-22].

The objectives of the present work were to study the physical stability, rheological and microstructural behaviour of W/O/W emulsions formulated with a “green” solvent (α -pinene) as dispersed phase and gellan gum as thickener, as a function of the mass ratio of two amphiphilic copolymers (AtlasTM G-5000 and AtloxTM 4912) used as emulsifiers.

2. MATERIALS & METHODS

2.1. *Materials*

Commercial low-acyl gellan gum, Kelcogel F type was used as supplied by CP Kelco Company (San Diego, USA) The final concentration of polysaccharide in the emulsion was 0.4 wt%. 0.1 wt% sodium azide was added to the final formulation to prevent the growth of microorganisms.

The organic solvent used was rectified α -pinene Leavo 95, an extremely non-polar solvent, which was supplied by Destilaciones Bordas-Chinchurreta S.A. (Sevilla, Spain). Its density (20° C) is 898 Kg/m³, its refractive index is 1.464 and its boiling point is around 67.5°C.

AtlasTM G-5000 (hydrophilic AB block copolymer, HLB: 16.9) and AtloxTM 4912 (hydrophobic ABA block copolymer, HLB:5.5) surfactants were supplied by Croda Iberica S.A. (Barcelona, Spain).

In addition, the Dow Corning^R MD 10 defoaming agent supplied by Dow Corning was used.

2.2. *Methods*

Each multiple emulsion studied was denominated as E(2)30/X/Y where 30 indicates 30 wt % of α -pinene, X is wt % of AtlasTM G-5000 and Y is wt % of AtloxTM 4912. The

total concentration of copolymers was 3.0 wt %, although the ratio Atlas™ G-5000 to Atlox™ 4912 was varied.

The gellan gum solution was prepared using a protocol described in previous paper of this group [9] and the emulsions were prepared as follows.

1.- The components of the aqueous phase (Atlas™ G-5000 copolymer, defoaming agent and water) were mixed using a magnetic stirrer (Hotplate stirrer [SB162-3], Stuart) in order to achieve an homogeneous phase. Likewise, the components of the oil phase (Atlox™ 4912 and α -pinene) were also mixed by means of the magnetic stirrer.

2.- Subsequently, the oil phase was slowly added to the aqueous phase to prepare the emulsion with rotor-stator device (Silverson L4RT homogenizer equipped with an emulsor screen) at 10600 r.p.m. for 150s.

3.- The pre-emulsion obtained was passed through a high-pressure valve homogenizer (FT9 Homogeniser, Armfield) three times at $6.9 \cdot 10^3$ kPa. The pre-emulsions were prepared in batches of 200 g.

4.- Finally, this emulsion was mixed with an equal amount of 0.8 wt % gellan gum aqueous dispersion. For this purpose, the Silverson L4RT was used at 10600 r.p.m. for 60s.

The methods used to characterize the emulsions studied are described below.

2.2.1. Confocal laser scanning microscopy

Emulsions were analysed by means of a Leica SPE confocal laser scanning microscope (CLSM). Red Nile dye was used to stain the oil phase and Blue Nile dye was utilized for aqueous phase. The samples were scanned at room temperature, using 60x oil immersion objective lens.

2.2.2. Electrolytic conductivity measurements.

Electrolytic conductivity measurements were performed at room temperature ($\sim 25^\circ\text{C}$) with an EC-Meter BASIC 30 (Crison) conductimeter in order to determine the nature of the external phase of the emulsions (Table 1).

2.2.3. Droplet size distribution

The mean droplet diameter of the emulsions was measured using a laser diffraction instrument (Mastersizer Hydro2000, Malvern Instruments, UK). The refraction and absorption indexes used were 1.464 and 1, respectively.

2.2.4. Oscillatory shear tests

Oscillatory stress sweeps were performed in the (0.01-3) Pa range at a fixed frequency (1 Hz).

Frequency sweep tests (from 3 to 0.01 Hz) were run by selecting a stress amplitude within the linear viscoelastic range.

A controlled-stress AR1000 rheometer with an aluminium cone-plate sensor (60 mm diameter, angle: 1°) of low inertia was used. Equilibration time prior to rheological tests was 600s.

2.2.5. Steady shear flow tests

The flow behaviour was carried using a serrated plate-plate sensor (40mm, gap: 1mm). Three different segments have been used to increase the number of data points along the flow curve: 1) From 0.5 to 10 Pa; 2) From 10.5 to 50 Pa; 3) From 50.5 to 70 Pa.

All rheological measurements were performed at $20^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$, using a temperature controller. The results represent the mean of three measurements.

3. Results and Discussion.

3.1. Microstructural characterisation

Figures 1, 2 and 3 illustrate the micrographs obtained by CLSM of three selected emulsions containing amphiphilic copolymers. The first of them (Figure 1) shows the pre-emulsion containing 1.5 wt% Atlas G-5000TM and 1.5 wt % Atlox 4912TM in the final formulation. In order to obtain this micrograph Blue Nile dye was used to stain the aqueous phase. It must be highlighted that the amount of pre-emulsion corresponded with 50 wt% of the final emulsion. This means that the preponderant phase in pre-emulsion was oil phase (60 wt%). This fact, along with Figure 1 and the pre-emulsion electrolytic conductivity data (Table 1), supported the fact that a W/O emulsion was

formed during the first step process by using a rotor-stator homogenizer. Micrographs showed in the Figures 2 and 3 demonstrated the formation of multiple emulsions even for the emulsion containing only the most hydrophilic copolymer, E(2)30/3/0 (Figure 2a). This fact can be attributed to the use of commercial copolymers with a broad molecular weight distribution such that the most hydrophobic fractions may promote the stabilisation of water droplets in the primary W/O emulsion. Figure 1 shows globules forming a network of entangled flocs which are trapped in the viscous continuous phase formed by gellan gum in the external phase. The presence of this hydrocolloid in the continuous phase provided a long-term physical stability (Figure 3b). Interestingly, the emulsion free of AtlasTM G-5000, E (2)30/0/3; i.e. the emulsion formulated with the lowest HLB copolymer (AtloxTM 4912), did not present W/O/W multiple emulsion structure, but O/W due to dilution effect of the gellan dispersion (Figure 3a). In addition, Figure 3a demonstrated that the lack of the most hydrophilic copolymer resulted in weak droplet interfaces which were prone to be broken by just placing the emulsion between the slide and cover slip of the microscope.

As abovementioned, the nature of the external phase of the emulsions was determined by means conductivity measurements (Table 1). The α -pinene presented a negligible conductivity. In contrast, the AtlasTM G-5000/water solution showed much higher conductivity than distilled water as reported in a previous work [17], demonstrating that the AtlasTM G-5000 commercial copolymer contained some charged groups. For instance, the conductivity of a water solution containing 1.8 wt % AtlasTM G-5000 was 123 μ S/cm. Table 1 showed the conductivities of pre-emulsions and final emulsions of three selected formulations. As can be observed, the conductivity values of pre-emulsions were negligible, indicating the existence of an oil-based continuous phase, whereas those of final emulsions were much higher and even greater than the conductivity of the AtlasTM G-5000 solution. This fact is likely due to the anionic nature of gellan gum and the overwhelming presence of water in the external phase.

Figure 1

Figure 2

Figure 3

Table 1

3.2. Globule size distribution (GSD)

As can be observed in the Figure 4, the right tail of the globule size distributions of emulsions aged for 2 days slightly shifted to higher sizes with decreasing values of Atlas™ G-5000/Atlox™ 4912 mass ratio, R, in emulsions formulated with both copolymers. Therefore the distributions became slightly more polydispersed as supported by the increasing values of the span (Table 2a) as R dropped. The Sauter mean diameter did not significantly change while the volume mean diameter showed a tendency to increase which was hardly significant at $R = 0.2$. Apart from that, the most dramatic effect was caused by the lack of the most hydrophilic copolymer in the formulation, since the whole distribution markedly shifted to the right, which logically resulted in a drastic increase in the mean diameters. It must be noted that the multiple emulsion containing only Atlas™ G-5000 as surfactant (the copolymer of HLB, 16.9) exhibited much smaller mean diameters than that containing just Atlox™ 4912, whose HLB is 5.5. The hydrophilic copolymer Atlas™ G-5000 is able to promote steric interactions among droplets, preventing the occurrence of significant coalescence. The results obtained showed that the inclusion of Atlas™ G-5000 was essential for the preparation of an acceptable emulsion. In fact, the presence of just 0.5 wt % Atlas™ G-5000 in the emulsion decreased the volume mean diameter by 7 times. The relevant performance of this copolymer is based on its ability to structure water in the continuous phase by forming hydrogen bonds thanks to its ethylene oxide groups. (Table 2a). In any case, the globule/droplet size distribution of all the emulsions studied remained quite stable between 2 and 15 days (Figure 4, Table 2).

Figure 4 (GSD)

Table 2 a (GSD)

3.3. Rheological Characterization

3.3.1. Oscillatory shear tests

The values of critical stress and strain locating the onset of non-linear oscillatory were not sensitive either to changes in formulation of emulsions containing both copolymers or aging time from 2 to 15 days.

Table 2b

Figure 5

Figure 5 illustrates the mechanical spectra of emulsions after 2 days of aging time. All emulsions showed values of the storage modulus (elastic component), G' , above the loss modulus (viscous component), G'' . Both G' and G'' increased with parameter R for emulsions containing both copolymers. The emulsion formulated with only AtloxTM 4912 copolymer exhibited the lowest viscoelastic modulus values, while the emulsion containing only AtlasTM G-5000 showed similar values to the emulsion with R equal to 2.

Further analysis of the mechanical spectra revealed the occurrence of two groups of emulsions as a function of dynamic modulus values. On the one hand, we define a first group including emulsions E(2)30/3/0, E(2)30/2.5/0.5 and E(2)30/2/1 (emulsions containing a high concentration of AtlasTM G-5000, $R \geq 2$). This group was characterized by mechanical spectra which were practically parallel to each other and similar values of dynamic viscoelastic modulus, suggesting that these emulsions possessed the same colloidal structure. On the other hand, a second group involved emulsions with $R \leq 1.0$ (E(2)30/1.5/1.5, E(2)0.5/2.5 and E (2)30/0/3). The mechanical spectra of this group exhibited G' and G'' values markedly lower than those of emulsions with $R \geq 2$. Yet, decreasing the AtlasTM G-5000 concentration to 1.5 wt% provoked an important decrease in the viscoelastic moduli. In addition, when AtlasTM G-5000 dropped to a critical concentration of 0.5 wt % ($R= 0.2$), a change in the shape of the G'' curve was observed, indicating that this concentration was critical for a change in the emulsion microstructure. It worth to note that the lack of AtlasTM G-5000, $R=0$, resulted in a mechanical spectrum to some extent similar to the emulsion with 0.5 wt% Atlas G-5000 ($R= 0.2$). However, the viscoelastic response of the emulsion formulated with only Atlox 4912 ($R= 0$) was consistent with a faster relaxation process (more fluid-like behaviour) than for the rest of emulsions studied.

With regards to the effect of aging time (data not shown), it must be mentioned pre-emulsions were destabilized in five hours by a creaming process. Additionally, in the emulsions with $R \geq 2$ did not exhibit changes either in the curve shapes of mechanical spectra or their G' and G'' values, indicating the lack of destabilization phenomena

(Figure 6). On the contrary, emulsions with R values between 0.2 and 1 showed an increase in both viscoelastic moduli. This along with the fact that the mean diameters did not significantly change with aging time pointed to the onset of destabilization by creaming (Figure 7). This could be explained considering that the Atlas™ G-5000 copolymer is able to promote steric interactions between globules due to its ability to form hydrogen bonds with the water in the continuous phase. The reduction of its concentration must decrease such interactions, weakening the network of globules. This, in turn, must accelerate the creaming process caused by the density difference between the α -pinene and aqueous phase. The progressive accumulation of globules in the upper part of the sample provoked an increase in the viscoelastic moduli. Conversely, the lack of the hydrophilic copolymer provoked a dramatic change in the governing destabilization mechanism. Yet, the emulsion formulated with Atlox™ 4912 copolymer, as the only emulsifier, showed lower values of both G' and G'' with aging time. This evolution points to the occurrence of some coalescence, demonstrating that oscillatory shear is a very sensitive methodology to detect structural changes in emulsions as demonstrated in other studies [23-26]. It must be considered that the occurrence of coalescence could hardly be observed by laser diffraction since the increase in volume mean diameter was not statistically significant (Table 2).

Figure 6

Figure 7

3.3.2. Flow Curves.

Flow curves are plotted in Figure 8. In order to take into account the distribution of shear rates along the parallel plate sensor radius, viscosity values were corrected [27]. The samples studied showed shear thinning behavior after aging for 2 days as well as for 15 days.

$$\eta_{\text{corr}}(\dot{\gamma}_R) = \eta_{\text{newt}} \cdot [1 + m/4] \quad (1)$$

where, $\eta_{\text{newt}} = (2 \cdot H / (\pi \cdot R^4)) \cdot (M / \Omega)$; $m = d(\log \eta_{\text{newt}}) / d(\log \dot{\gamma}_R) = n - 1$

$\dot{\gamma}_R$ is the border shear rate; H is gap among plates; Ω is the angular velocity; R is radius of plates, M is the torque applied and η_{newt} is the viscosity calculated at the border of the parallel plate geometry.

Figure 8

Table 3

As can be observed in the Figure 9, the flow curves showed very shear thinning behaviour for all emulsions studied. In addition, the emulsions formulated with the higher R values exhibited the slightly greater values of viscosity, accordingly to results provided by mechanical spectra.

Regarding aging time, there were no changes in emulsions with $R \geq 2$. Nevertheless, emulsions with R ranging from 0.2 to 1 showed an increase in viscosity at the lower shear rates. Even though the differences observed were not statistically significant, the results obtained were consistent with the occurrence of incipient creaming as supported by mechanical spectra results.

Flow curves fitted the power law equation (2):

$$\eta = \eta_1 \cdot \dot{\gamma}^{(n-1)} \quad (2)$$

where η_1 is the viscosity calculated at 1 s^{-1} of shear rate and n is nowadays called the power law index [28]. The fitting parameters are shown in Table 3.

An analysis of these parameters revealed that there was no tendency with the mass ratio R. Likewise, there was no influence of the aging time on flow curves since the shear effects on the emulsion microstructure were more important than the microstructural changes associated to different formulations or aging effects, which can be readily detected and monitored by small amplitude oscillatory shear tests.

4. Conclusions.

Multiple emulsions containing 30 wt% α -pinene as dispersed phase prepared with 3 wt% amphiphilic copolymers mixture as surfactants and 0.4 wt% gellan gum as stabilizer have been successfully obtained by rotor stator homogenizer followed by high pressure valve homogenizer.

Confocal laser scanning microscopy micrographs revealed that it was necessary to include 0.5% of Atlas™ G-5000 in the formulation to obtain multiple emulsions.

There was no significant dependence of the Atlas™ G-5000/Atlox™ 4912 mass ratio on mean diameters except when the most hydrophilic copolymer was removed from the formulation. The lack of Atlas™ G-5000 caused that the globule size distribution dramatically shifted towards much larger sizes.

All emulsions exhibited viscoelastic properties with the elastic component above the viscous one in the whole frequency range studied and they presented a clear shear thinning flow behavior. Flow curves were not sensitive to either the mass ratio of copolymers used or the changes produced with aging time. Conversely, the mechanical spectra obtained by small amplitude oscillatory shear turned out to be quite sensitive to both variables. Multiple emulsions formulated with the two copolymers studied, showed mechanical spectra with greater storage and loss modulus values as the Atlas™ G-5000/Atlox™ 4912 mass ratio was increased. This fact was due to a reinforcement of the network of entangled globules as a result of the higher concentration of the more hydrophilic copolymer, Atlas™ G-5000, which is able to improve the steric interactions between globules. Regarding the aging time, the emulsions with $R \geq 2$ did not show influence on the mechanical spectra. However, those with $0.2 < R < 1$ exhibited an increase in the viscoelastic modulus which indicated the onset of destabilization by creaming. Conversely, the mechanical spectrum of the emulsion formulated only with Atlox™ 4912 showed a decrease of the viscoelastic modulus with aging time revealing the occurrence of some coalescence.

From the rheological results, globule size distribution and microstructural information obtained we draw the conclusion that the best emulsions were obtained when the Atlas™ G-5000/Atlox™ 4912 mass ratio varied between 2 and 5.

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Figure captions

Figure 1. Confocal laser scanning micrographs of W/O pre-emulsion corresponding to E(2) 30/1.5/1.5 W/ α -pinene/W emulsions.

Figure 2. Confocal laser scanning micrographs of W/ α -pinene/W emulsions: (a) E(2)30/3/0; (b) E(2)30/2/1. Aging time: 2 days. Room temperature.

Figure 3. Confocal laser scanning micrographs of W/ α -pinene/W emulsions: (a) E(2)30/0/3, aging time: 2 days; (b) E(2)30/2/1, aging time: 15 days. Room temperature.

Figure 4. Influence of the copolymer ratio on the oil globule size distribution of W/ α -pinene/W emulsions: a) aging time: 2 days; b) aging time: 15 days. Room temperature.

Figure 5. Mechanical spectra of W/ α -pinene/W emulsions as a function of the copolymer ratio used as emulsifier. Aging time: 2 days. T=20°C.

Figure 6. W/O pre-emulsion photographic corresponding to E(2) 30/1.5/1.5 W/ α -pinene/W emulsions at five hour of aging time.

Figure 7. Photographic corresponding to E(2) 30/2.5/0.5 W/ α -pinene/W emulsions.

Figure 8. Photographic corresponding to E(2) 30/0.5/2.5 W/ α -pinene/W emulsions.

Figure 9. Flow curves of W/ α -pinene/W emulsions as a function of the copolymer ratio used as emulsifier. (a) Aging time: 2 days; (b) Aging time: 15 days. T=20°C.

Table captions

Table 1. The electrolytic conductivity of multiple emulsions for selected pre-emulsions and final emulsions. Tests done at room temperature.

Table 2. . Influence of the copolymer ratio used as emulsifier of multiple emulsions on the Sauter (D[3,2]) and volumetric (D[4,3]) mean diameters and the span. a) aging time: 2 days; b) aging time: 15 days. Tests done at room temperature.

Table 3. Influence of the copolymer ratio used as emulsifier of multiple emulsions on the fitting parameters of the power lay equation for flow curves at 20°C. SD is standard deviation of the mean (n = 3) for η_1 and (n-1) as slope of power law plot: a) aging time: 2 days; b) aging time: 15 days.

Tables

Table 1. The electrolytic conductivity of multiple emulsions for selected pre-emulsions and final emulsions. Tests done at room temperature.

	Pre-emulsion conductivity/ $\mu\text{S}\cdot\text{cm}^{-1}$	Final emulsion conductivity/ $\mu\text{S}\cdot\text{cm}^{-1}$
E(2)30/3/0	0.05	646
E(2)30/1.5/1.5	0.04	653
E(2)30/0/3	0.04	635

Table 2. . Influence of the copolymer ratio used as emulsifier of multiple emulsions on the Sauter (D[3,2]) and volumetric (D[4,3]) mean diameters and the span. a) aging time: 2 days; b) aging time: 15 days. Tests done at room temperature.

Table 2a

	R=Atlas TM G-5000/Atlox TM 4912 mass ratio	Aging time			Aging time		
		2days			15 days		
		D [3,2] (μm)	D [4,3] (μm)	Span	D [3,2] (μm)	D [4,3] (μm)	Span
E(2)30/3/0	-	2.59	3.12	1.20	2.69	3.46	1.43
E(2)30/2.5/0.5	5	2.66	3.24	1.23	2.74	3.34	1.22
E(2)30/2/1	2	2.38	3.11	1.36	2.62	3.31	1.35
E(2)30/1.5/1.5	1	2.52	3.46	1.43	2.67	3.54	1.58
E(2)30/0.5/2.5	0.2	2.72	4.00	1.73	2.80	4.48	1.90
E(2)30/0/3	0	12.11	29.20	1.16	13.30	30.82	1.29

Standard deviation of the mean (4 replicates) for D[3,2] < 5%, for D [4,3] < 8% and for span < 5%

Table 3. Influence of the copolymer ratio used as emulsifier of multiple emulsions on the fitting parameters of the power law equation for flow curves at 20°C. SD is standard deviation of the mean ($n = 3$) for η_1 and $(n-1)$ as slope of power law plot: a) aging time: 2 days; b) aging time: 15 days.

Table 3a

	R=Atlas 5000™/Atlox 4912 ratio	G- mass	η_1 (Pa·s)	SD_k	n-1	SD_{n-1}	n	R²
E(2)30/3/0	-		5.90	0.11	-0.85	-	0.15	0.996
E(2)30/2.5/0.5	5		5.98	0.75	-0.82	0.002	0.18	0.991
E(2)30/2/1	2		4.98	0.08	-0.76	-	0.24	0.997
E(2)30/1.5/1.5	1		3.92	0.03	-0.72	-	0.28	0.999
E(2)30/0.5/2.5	0.2		4.17	0.05	-0.77	-	0.23	0.998
E(2)30/0/3	0		6.21	0.07	-0.77	-	0.23	0.998

Table 3b

	R=Atlas 5000™/Atlox 4912 ratio	G- mass	η_1 (Pa·s)	SD_k	n-1	SD_{n-1}	n	R²
E(2)30/3/0	-		5.23	0.12	-0.83	0.004	0.17	0.999
E(2)30/2.5/0.5	5		5.3	0.14	-0.81	0.01	0.19	0.999
E(2)30/2/1	2		5.54	0.02	-0.81	-	0.19	0.999
E(2)30/1.5/1.5	1		4.14	0.01	-0.79	-	0.21	0.999
E(2)30/0.5/2.5	0.2		5.43	0.02	-0.87	-	0.13	0.999
E(2)30/0/3	0		5.95	0.09	-0.70	-	0.30	0.997

