Research Article

Spontaneous nano-emulsification with tailor-made amphiphilic polymers and related monomers

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Abstract

In general, nano-emulsions are submicron droplets composed of liquid oil phase dispersed in liquid aqueous bulk phase. They are stable and very powerful systems when it regards the encapsulation of lipophilic compounds and their dispersion in aqueous medium. On the other hand, when the properties of the nano-emulsions aim to be modified, e.g. for changing their surface properties, decorating the droplets with targeting ligands, or modifying the surface charge, the dynamic liquid/liquid interfaces make it relatively challenging. In this study, we have explored the development of nano-emulsions which were not anymore stabilized with a classical low-molecular weight surfactant, but instead, with an amphiphilic polymer based on poly(maleic anhydride-alt-1-octadecene) (PMAO) and Jeffamine®, a hydrophilic amino-terminated PPG/PEG copolymer. Using a polymer as stabilizer is a potential solution for the nano-emulsion functionalization, ensuring the droplet stabilization as well as being a platform for the droplet decoration with ligands (for instance after addition of function groups in the terminations of the chains). The main idea of the present work was to understand if the spontaneous emulsification—commonly performed with nonionic surfactants—can be transposed with amphiphilic polymers, and a secondary objective was to identify the main parameters impacting on the process. PMAO was modified with two different Jeffamine®, additionally different oils and different formulation conditions were evaluated. As a control, the parent monomer, octadecyl succinic anhydride (OSA) was also modified and studied in the similar way as that of polymer. The generated nano-emulsions were mainly studied by dynamic light scattering and electron microscopy, that allows discriminating the crucial parameters in the spontaneous process, originally conducted with polymers as only stabilizer.

Keywords: Spontaneous emulsification; PMAO; nano-emulsion; surfactant; Jeffamine.

1. Introduction

Nano-emulsions are fine, stable, and in general optically clear and translucent oil-in-water or water-in-oil dispersions, stabilized by a nonionic surfactant. Droplets size range below 200-300 nm (N. Anton & Vandamme, 2009; Mahato, 2017; Mason, Wilking,
Meleson, Chang, & Graves, 2006; McClements, 2012; Solans, Morales, & Homs, 2016). The small droplet size results in long-term physical stability of nano-emulsions, because it impairs the destabilization phenomena like sedimentation, creaming and coalescence (Singh et al., 2017; Solans et al., 2016). Nano-emulsions can be administered by various routes like oral, ocular, topical, intravenous, pulmonary and intranasal route because of the compatibility of their physico-chemical properties with the different administration routes, as well as the possibility of rendering them into different kinds of dosage forms, like creams, gels, liquids and aerosols (Ammar, Salama, Ghorab, & Mahmoud, 2009; Hussain et al., 2016; Khani, Keyhanfar, & Amani, 2016; Nasr, Nawaz, & Elhissi, 2012; Pawar et al., 2014; Singh et al., 2017). The nano-emulsions are thermodynamically unstable and kinetically stable systems. Unlike nanostructures of microemulsions, the nano-emulsion droplets can remain stable in stress conditions as well like temperature and concentration (e.g. dilution) modifications (Nicolás Anton & Vandamme, 2011; Lefebvre et al., 2017; Singh et al., 2017). The methods used to prepare nano-emulsions can be classified into two main types, (i) high-energy methods and (ii) low-energy methods (Anton, Benoit, & Saulnier, 2008). Over the past twenty five years, the research interest in the development of low-energy methods has increased because of their numerous advantages like smaller droplet sizes, low cost, high formulation yields, encapsulation of fragile active molecules, potential industrial scale up and wide range of applications (food, cosmetic and pharmaceutical industry). The low-energy methods divert the intrinsic physicochemical properties of the surfactants, co-surfactants and excipients in the formulation, leading to the generation of stable emulsion droplets in the nanometric range (N. Anton & Vandamme, 2009). A special character of these methods is the utilization of energy stored in the system to produce ultra-fine droplets.

Among the various low-energy methods, spontaneous emulsification phenomenon has been the focus of attention due to its diverse applications and easy industrial scaling-up, particularly in pharmaceutical and cosmetics field. The spontaneous emulsification is a very simple method in which an aqueous phase is mixed with an oil phase—composed of a mixture of oil, surfactant and/or water miscible solvent. Various mechanisms related to the droplet formation process have been proposed in the literature, and the most important and well-documented methods are explained by the creation, during the spontaneous emulsification, of interfacial turbulence, development of transient values of interfacial tensions, diffusion and stranding, Marangoni flow and Rayleigh-Taylor instability (López-Montilla, Herrera-Morales, Pandey, & Shah, 2002; Solans et al., 2016). However the general mechanism is the rapid displacement of a solvent, from the oil phase to the aqueous phase that induces turbulence and super saturation at the oil/water interface (Miller, 1988; Solans et al., 2016). The major drawback associated with this method is in fact the use of the solvent itself, like ethanol, acetone, that can limit the application compatibilities and make the whole process complicated—e.g. if including solvent evaporation step. In this context, our group has already shown that non-ionic surfactants themselves can play the role of the displacing species in the spontaneous emulsification process, following an universal mechanism related to the sudden displacement of the surfactants from the oil to the aqueous phase, due to the sudden change of surfactant solubility (N. Anton & Vandamme, 2009).

In the context of the control of the chemical composition of the surface, we have reported preliminary studies that describe original methods to modify the water/oil interface, and reinforce the droplet with in situ synthesis of silica shell at the droplet interfaces (Attia et al., 2015), or by anchorage of polymeric amphiphiles (Attia et al., 2017). However, these methods still use surfactants to help the nano-droplet formation, and in some extents, it could constitute a limitation—in term of biocompatibility, toxicity, development of incompatibilities with time (Hou & Xu, 2016; Kaci et al., 2016; Nehilla, Bergkvist, Popat, & Desai, 2008; Sahoo, Panyam, Prabha, & Labhasetwar, 2002; Sheibat-Othman & Bourgeat-Lami, 2009)—or in term of efficiency of the surface modification.

To deal with this aspect of the nano-emulsion formulation related to surface modification, we propose in the current study, investigating how nano-emulsions can be formulated by low-energy emulsification when the nonionic surfactants are totally replaced for an amphiphilic polymer. The most innovative aspect lies in the fact that we describe for the first time that spontaneous nano-emulsification can be successfully performed using a macromolecule migrating from the oil phase to the liquid/liquid interface. In fact, the formulation of nano-emulsions stabilized by polymers presents numerous advantages respective to the conventional surfactant-stabilized droplets, mainly the surface functionalization—related to the chemical function present at the termination of the hydrophilic parts of the polymer—and secondly the droplet stability.

This study begins with the design and synthesis of amphiphilic polymers, specially tailored to exhibit an important surface-active property, followed by the evaluation of its ability to generate nano-emulsions through the spontaneous emulsification method. The synthesis of amphiphile polymer is based on the modification of poly(maleic anhydride-alt-1-octadecene) (PMAO), which is a hydrophobic polymer having aliphatic octadecene chains (C18) with reactive anhydride functions. Herein we start from a former study (Attia et al., 2017) on which we showed their solubility in the oil core and their ability to migrate at the interfacial layer driven by the reactivity of its anhydride function. PMAO is a low-cost lipophilic polymer, for which the monomeric group is composed of aliphatic octadecene chain and a reactive cyclic anhydride function. Our objective was to modify the PMAO by performing a systematic reaction of Jeffamine® polyetheramine (polyethylene glycol / polypropylene glycol copolymer) on each anhydride function, in order to generate an octadecene / Jeffamine® pattern on each monomer element. As a result, it gives rise to a polymer perfectly designed to stabilize the oil / water interfaces, similarly to a high number of nonionic surfactants assembled together. The different
formulations, chemical and physico-chemical parameters inherent to the emulsification process were studied, and their impact on the properties, size and polydispersity of the droplets was carefully analyzed. In addition, Jeffamine® 1000 / 2000 was chosen because of the high amount of PEG in the chain composition. This will result in high interfacial stabilization creating steric repulsion among the neighboring nano-droplets (Hörmann & Zimmer, 2016), but also well-recognized to decrease the immune system uptake after in vivo administration, inhibiting opsonization and phagocytosis as observed with nano-emulsions (Cristina Fornaguera et al., 2015; Hörmann & Zimmer, 2016). In addition, and in order to compare the impact of the macromolecular structure, we have designed similar amphiphiles with J1000 and J2000, but using the parent monomer of PMAO, i.e. octadecyl succinic anhydride (OSA). The idea was to use it in place of PMAO based amphiphile, keeping the rest of the parameters constant, to observe in parallel the relative abilities of the monomer and polymer to form nano-emulsions and also their impact on the size of the nano droplets. The resulting species exhibit a structure close to nonionic surfactants, making a bridge between modified PMAO and literature.

Beyond such a pioneer description of spontaneous nano-emulsification performed using only polymeric molecule—without low molecular weight surfactants—this study proposes several new ideas on the potential route that can serve to the functionalization of emulsion, that is to say functionalizing dynamic liquid / liquid interfaces. As an example, a proportion of the Jeffamine® herein used can be terminated with another reactive group and finally covalently linked with, for instance, a ligand. Emulsions droplets constitute a high-potential nano-carrier for hundred applications owing to their reservoir capability, with a main drawback in their surface modification and stability: the solution herein presented is an illustration that simple solutions can exist, can be easily developed and transposed to large-scale production.

2. Materials and Methods

2.1. Chemicals

Poly (maleic anhydride-alt-1-octadecene) (PMAO), triethylamine (TEA), tetrahydrofuran (THF) octadecyl succinic anhydride (OSA) and dichloromethane (DCM) were purchased from Sigma Aldrich. Jeffamine® M-1000 (J-1000) and Jeffamine® M-2070 polyetheramine (J-2000) were kindly offered by Huntsman corporation (Texas, U.S.A.). Medium chain triglycerides (Labrafac WL®) and Pluronic were purchased from Gattefossé (Saint Priest, France). The rest of the reagents and solvents used were of analytical grade.

2.2. Polymer chemical modification

The global synthesis is illustrated in Fig. 1 (a). To a solution of Jeffamine® (J-1000 or J-2000, 2.85 mmol) in THF (about 7 mL) was added PMAO (1.00 g, 2.85 mmol based on repeating unit) Then the volume of THF was completed up to 50 mL. After total solubilization of the PMAO, 1.2 mL of TEA (3 equivalents) was added into the solution that was warmed at 80°C and allowed to stir at this temperature overnight. Next, the THF was evaporated using rotavapor, and the resulting product was dried under vacuum.

Jeffamine® M-1000 and M-2070 polyether amine (J-1000 and J-2000) are monoamines based on a copolymer backbone with an average molecular weight of 1000 and 2000, respectively. Their propylene oxide / ethylene oxide (PO/EO) molar ratio is 3/19 and 10/31, respectively.
2.3. **Monomer chemical modification**

The global synthesis is illustrated in Fig. 1 (b). The experimental procedure followed was strictly the same as described above for the polymer. After solvent evaporation by rotary evaporator, the obtained product was dissolved in DCM and then washed using a separatory funnel after mixing different aqueous phases (i) first 1M HCl aqueous phase, (ii) then with saturated NaCl solution, and (iii) finally dried by using sodium sulfate. The mixture was filtered under filter paper, and DCM evaporated using rotary evaporator to collect the final product.

2.4. **$^1$H-NMR Analysis**

To confirm the structure of the amphiphilic polymers / monomers, 25 mg of these products were dissolved in 600 µL of deuterated chloroform (CDCl$_3$) and 1H-NMR spectrum was studied with a Bruker Top Spin 3.0 operating at 400 MHz. The chemical shifts (δ) were expressed in parts per million.

2.5. **Preparation of Nano-emulsions**

First of all, both oil and aqueous phases were prepared separately and heated at 90°C for 20 min. Oil phase consists of monomer or polymer dissolved in oil (medium chain triglycerides, Labrafac WL$^\text{®}$), while the aqueous phase was distilled water. The second step is the spontaneous emulsification process (N. Anton & Vandamme, 2009): aqueous and oil phases were rapidly mixed together and homogenized by vortex for 2 min, giving rise to the formation of nano-emulsions (Fig. 2).

2.6. **Dynamic light scattering**

The hydrodynamic diameters of the nano emulsion droplets were measured, in triplicate, by dynamic light scattering using a Malvern Nano ZS instrument, equipped with a helium-neon laser 4 mW operating at 633 nm, with the scatter angle fixed at 173° and the temperature was maintained at 25°C. The polydispersity index (PDI) was also measured which shows the quality of the dispersion. Generally, the PDI values $\leq$ 0.2 indicate good monodispersity and a good quality of the nano-emulsions.

2.7. **Microscopic observations**

The morphology and structure of the nano droplets formed were studied by transmission electron microscopy (TEM). The nano-emulsion samples were diluted (1/100) with Milli-Q water. One drop of the diluted nano-emulsion was introduced on a carbon grid (carbon type A, 300 mesh, copper, Ted Pella Inc. Redding, PA) and dried at 40°C. The observations were carried out by a Philips Morgagni 268D electron microscope.

2.8. **Optimization of the formulation parameters**

Nano-emulsions were generated by the addition of aqueous phase into the oil phase. As it is an important parameter for common spontaneous emulsification of nano-emulsions with surfactant (surfactant-to-oil weight ratio, SOR expressed in percen-
similar parameter were herein defined when using the polymer: the polymer-to-oil weight ratio (POR, in %) -while SOR is used when using the monomer, similar to a surfactant. Impact of POR and SOR on size distribution and PDI were followed, keeping the rest of the parameters constant (temperature, mixing time, volume of aqueous phase). The experiments were performed using both the Jeffamine® derivatives (J1000 and J2000), grafted on both polymer and monomer.

2.9. Effect of nature of oil on emulsification

To study the effect of nature of oil on the formation of nano-emulsions, size and polydispersity, the formulation was followed with different oils, soy bean oil, fish oil and Plurol oleic. Based on the results obtained with different POR and SOR ratios, two values were selected: 50% and 80%. The rest of the parameters were kept constant and nano-emulsions were prepared using the same procedure described above.

3. Results and discussion

First, the amphiphilic monomers and polymers were synthesized from OSA and PMAO respectively, by mean of addition of Jeffamine® 1000 or 2000 in the presence of

Figure 3: Superimposed NMR spectra of amphiphilic polymers and PMAO (a) and amphiphilic monomers and OSA (b) showing the shifts of the signals and the increasing amount of PEG and PPG compared to the starting materials. The NMR spectra were normalized to the CH₃ signal of the C₁₈ chain at 0.8-0.9 ppm. The spectra were cropped for a better visibility.
triethylamine (Fig. 1). The obtained amphiphiles were characterized by 1H NMR spectroscopy (Fig. 3). The spectra showed significant shifts of characteristic signals compared to the starting materials along with increasing PEG and PPG signals from the grafted Jeffamine®, thus proving the chemical efficient modifications.

Nonionic surfactants are used in the preparation of nano-emulsions and the literature has extensively shown their crucial role in the emulsification processes (N. Anton, Akram, & Vandamme, 2018; N. Anton & Vandamme, 2009). After mixing homogeneously the {oil + surfactant} phase, the spontaneous emulsification process was indeed driven by the turbulent solubilization of the surfactant by the aqueous phase and during homogenization of these three compounds. Making nano-emulsions using amphiphilic polymer as stabilizer –or core-shell nano-droplets– is a real advantage towards the classical surfactant based emulsions: at first sight the first advantage appears as an improvement of the droplet stability. In addition, and importantly, a second advantage lies in the potential functionalization of the droplet surface, much more strongly anchored when it is performed onto a polymeric shell, compared to surfactant anchorage lonely made by molecular adsorption. On the other hand, when making nano-emulsions by spontaneous emulsification is simple, the corresponding processes using polymers are not comparatively simple (N. Anton, Benoit, & Saulnier, 2008; Nehilla et al., 2008; Sahoo et al., 2002). Through this study, we propose to investigate whether spontaneous emulsification can be performed with an amphiphilic polymer as it is the case with common nonionic surfactants, but this time without surfactant. In is also important to note that such a formulation method, besides following a simple and scalable process, is able to produce ready-to-use suspensions, that is to say without need of further purification –e.g. for removing chemical precursors when compared with the in situ polymerization with residual monomer or initiator, or undesired by-products (Calderó, García-Celma, & Solans, 2011; C Fornaguera, Feiner-Gracia, Calderó, García-Celma, & Solans, 2016).

Both Jeffamine® polyetheramines (J-1000 and J-2000) used in this study are predominantly composed of PEG, making their structure highly similar to the polar head of nonionic surfactants generally used in the spontaneous nano-emulsification processes (N. Anton et al., 2018), and therefore should induce, similarly, efficient stabilizer properties of the species synthetized in the present study. In the case of nonionic surfactants, right after mixing, the water phase immediately and suddenly penetrate the oily phase to solubilize the amphiphilic molecules. Due to the sudden composition change, the physico-chemical properties of the global system undergoes a drastic change making the surfactant much more hydrophilic than before mixing –thanks to the effect of temperature and/or dilution (N. Anton & Vandamme, 2009; Nicolas Anton & Vandamme, 2011; Hörmann & Zimmer, 2016; Miller, 1988; Solans et al., 2016). The water breaks-up the oil phase and the surfactants are assumed to be shared between the water/oil interface and solubilized in water (Fig. 2). Regarding the amphiphilic polymer, the principle proposed is close, playing on its own partitioning between the oily and aqueous phases, before and after their mixing. The expected results would be the fractionation of oily phase by the water, generating nano-droplets, but with the difference –compared with nonionic surfactants– that the polymeric molecules stays trapped at the oil/water interface since it is not soluble neither in oil nor in water at room temperature, making a

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**Figure 4:** Spontaneous emulsification (a) PMAO-Jeffamine® (1000 or 2000), or (b) OSA-Jeffamine® (1000 or 2000), using medium chain triglycerides (Labrafac WL®) as oil phase. Effect of the formulation parameters polymer-to-oil weight ratio or surfactant-to-oil weight ratio (POR and SOR, respectively) on the resulting droplet size and polydispersity suspension (PDI are indicated in the graph for each point).
core-shell droplet structure at final (Fig. 2).

Figure 4 summarizes the main results obtained, comparing the nano-emulsification performed with polymer (PMAO) and monomer (OSA) for different size of Jeffamine® grafted (J1000 and J2000). Through the values of the droplet sizes and the PDI, these results give the efficiency of the process itself, along with a fine comparison of the different experimental configurations.

These results present the main polymer-oil and monomer-oil ratios allowing to getting the optimum conditions for the formation of stable nano-emulsions, with size in the desired range (i.e. below 300 nm) and good monodispersity. The first very important point to note when observing Fig. 4 owes to the fact that the spontaneous emulsification process without surfactant, i.e. only with amphiphilic polymer, works, and works according to a similar way to the ones generally observed with classical nonionic surfactants (N. Anton & Vandamme, 2009). Globally the results showed that increasing the amount of the polymer or surfactant induces a decrease in the size of the nano-suspensions. The results are in accordance with our hypothesis that our modified surfactant (OSA-Jeffamine®) or polymer (PMAO-Jeffamine®) plays the role of surfactant itself as it is described in literature, and as well, seems to induce a size control on the dispersion (Calderó et al., 2011; Lefebvre et al., 2017; Saberi, Fang, & McClements, 2013).

It is interesting to note that the best results arise for the polymer formulation: indeed, even if the size range are close when we compare PMAO and OSA, the main difference is given by both the data fluctuation –values of standard deviation–, and the PDI values. It follows therefore that the process with OSA-Jeffamine® is not reproducible and dispersion not monodisperse (PDI > 0.3-0.4), while PMAO-Jeffamine® are much more reproducible with PDI < 0.2. The best results were obtained (for both PMAO-J1000 and PMAO-J2000) for the values of POR equal to 80% and 90%, that generates nanodroplets having sizes lower than 300 nm and PDI < 0.2. The results are clear, and appear (i) much better with the polymeric compounds compared to the corresponding OSA surfactants, and (ii) slightly better with a longer Jeffamine® chain. In fact, this is a surprising point since nonionic surfactants are known for showing very good surface activity, and intuitively one can expect a better efficiency of the low molecular weight species in the spontaneous emulsification process compared to the polymers. However, the better reproducibility and monodispersity of the nanoparticles formed with the PMAO based disclosed a better interfacial synergy. Several explanations can be found: (i) the first comes in the fact that in general, “polar head” of nonionic surfactants are made with pure PEG (and not co-polymer PEG/polypropylene glycol) as it is the case here; even if the ratio is largely favorable to the proportion of PEG (19/3 and 31/10, as PEG/polypropylene ratios glycol for J-1000 and J-2000, respectively), the introduction of polypropylene glycol could impact their water solubility and thus the process efficiency. (ii) A second reason could come from the negatively charged carboxylate present on the amphiphile molecules once in aqueous solution– its impact on the interfacial behavior of the molecule does not appear negligible. In both cases, the better emulsification observed with the polymer could come from the global interfacial inertia of the molecule, higher with higher molecular weight, thus forming more stable interfaces when adsorbed at interface.

Based on these results, the next step we decided to investigate and optimize the impact of the nature of the oil on the spontaneous

Figure 5: Effect of nature of oil on the mean size of the nano-droplets generated by spontaneous emulsification, for (a) PMAO-J2000 and (b) OSA-J2000, and for two representative values of POR and SOR= 50% and 80%. In figure (b), SOR = 50% with Soybean oil is not present as no emulsion was obtained.
emulsification. In fact, we discussed above the fact that the affinity of the polymers (or of the surfactants) for the water phase —by changing the PEG length— importantly impacts on the emulsification process, but their affinity with the nature of the oil can also be affected. To this regard, representative compounds were selected as J-2000 derivatives of PMAO and OSA, with two representative values of POR and SOR: 50% and 80%. Then, several different oils were used instead of medium chain triglyceride (Labrafac WL\textsuperscript{®}), and the spontaneous emulsification process followed was strictly similar to the one described above. The mean sizes of the nano-droplets obtained are reported in Fig. 5.

It clearly appears that significant variations arise, especially impacted by the POR/SOR variations (confirming the results observed in Fig. 4). A slightly more regular behavior is provided by the polymer in the same line as that of the previous results. To summarize, the effect on the size of the polymer or surfactant-to-oil ratio is similar as the one observed with Labrafac WL\textsuperscript{®}, but the medium chain triglycerides still remain one of the best candidate, along with Plurol oleic (a lipophilic amphiphile molecule). Even if all these oily phases are able to fully solubilize PMAO and OSA modified Jeffamine\textsuperscript{®}, their impact on the emulsification processes indicate that their affinity —chemically speaking— is able to impact on the turbulent molecular migrations at the origin of the emulsifications.

An additional characterization technique, important in the formulation of nanoparticulate carriers, is their direct visualization in electron microscopy. The results are presented in Fig. 6, for the PMAO-J2000, POR = 80%. The first information is the confirmation of the very spherical morphology of the particles fabricated, and this clearly proves that the nano-emulsions have a capsular—core-shell—structure, since when it is classically stabilized with surfactant, the TEM measurement generally destroys the spherical structure (Attia et al., 2016) and make the nano-emulsions droplets spread and merge each other onto the carbon support. In this case, the very spherical shape is conserve, showing that the strong stabilization of the interface is effectively achieved by the precipitation of the polymer in the form of a shell. The second observation regards the sizes revealed by the TEM pictures: they appear in line with the ones shown with DLS in the previous figures, with an average value between 200 and 300 nm. In addition, such TEM pictures also give a visual illustration of the dispersity of the nano-emulsion suspension, showing the important presence of droplets much smaller than the average size of the log-normal distribution —in fact, as expected.

4. Conclusion

In this study, we have explored the development of nano-emulsions using an amphiphilic polymer, synthetized from PMAO on which Jeffamine\textsuperscript{®} was grafted. Nano-emulsions composed of dynamic liquid / liquid interfaces are powerful system, but suffer from difficulties to precisely control the chemical modification of their surface. Using a polymer as stabilizer —instead of nonionic surfactant— is a potential solution for this issue, ensuring the droplet stabilization as well as being a platform for the droplet decoration with cell targeting moiety like ligands or antibodies. The main idea of this investigation was to understand if the spontaneous emulsification —commonly performed with nonionic surfactants— can be transposed with amphiphilic polymers and compared to the same experiment conducted with the parent monomer of PMAO also modified with Jeffamine\textsuperscript{®}. A secondary objective was to identify the main parameters impacting on the spontaneous emulsification process. To this end, PMAO was grafted with two Jeffamines\textsuperscript{®} of different length (average Mw of 1000 and 2000) compared with surfactant made with the PMAO monomeric entity (OSA). The main results clearly show that the spontaneous emulsification process works, discriminating that the most efficient conditions were obtained with longer Jeffamine\textsuperscript{®} and the PMAO compared to OSA surfactant. During the spontaneous process, as soon as the aqueous phase comes in contact with the oily phase — containing the amphiphiles species—, the modified polymers rearrange themselves in order to displace and orientate the hydrophilic Jeffamine\textsuperscript{®} towards the aqueous phase, i.e. at water / oil interface. On the one hand, Jeffamines\textsuperscript{®} are covalently attached to the polymer backbone and thus to the nano-emulsion droplet, and on the other hand, these chains are available at the surface of the droplets, ensuring the stability of the nano-emulsions, and potentially can be functionalized. It was further observed that the size of the formed droplets could significantly vary with the change in the nature of the oil. Eventually, the spontaneous emulsification process is related to the affinities of the stabilizer, polymer or

![Figure 6: TEM analysis of PMAO-J2000 (POR = 80%) nano-emulsions.](image)
surfactant, for oil and water. In the case of polymer, the originality comes with its non-dispersion in water and precipitation in the interfacial region, forming a spherical shell shape, confirmed by TEM. To finish, this study may have important implications in the development of surfactant-free nano-emulsions, as well as in the development of functional emulsions and nano-emulsions, along with the fact that spontaneous emulsification has never been reported with polymer without surfactant.

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6. References


