Electrolytes as a tuning parameter to control nano-emulsion and nanoparticle size

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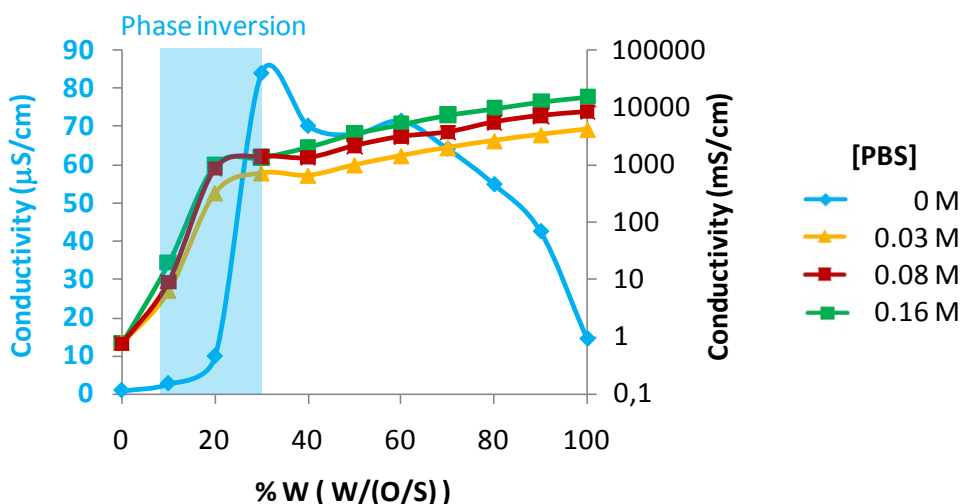
Figure S1: Conductivity of the dilution path of a nano-emulsions with an O/S ratio of 70/30, varying the electrolyte concentration of the aqueous phase (in different colors), as a function of aqueous phase percentage.

The measurements of conductivity as a function of the aqueous phase percentage enabled to confirm that an inversion of the phases took place for those nano-emulsions formulated with water as the aqueous phase, as previously demonstrated in other studies of our group (Fornaguera, 2015DXM; Calderó, 2011). However, for those nano-emulsions containing electrolytes in the aqueous phase, conductivity measurements were not useful to demonstrate an inversion of the phases, since the addition of aqueous phase increases the conductivity due to an increase on the electrolyte concentration. For this reason, the inversion of the phases was qualitatively assessed by means of visual observation of the diffusion of a hydrophilic and a hydrophobic dye, as shown in Figure S2.
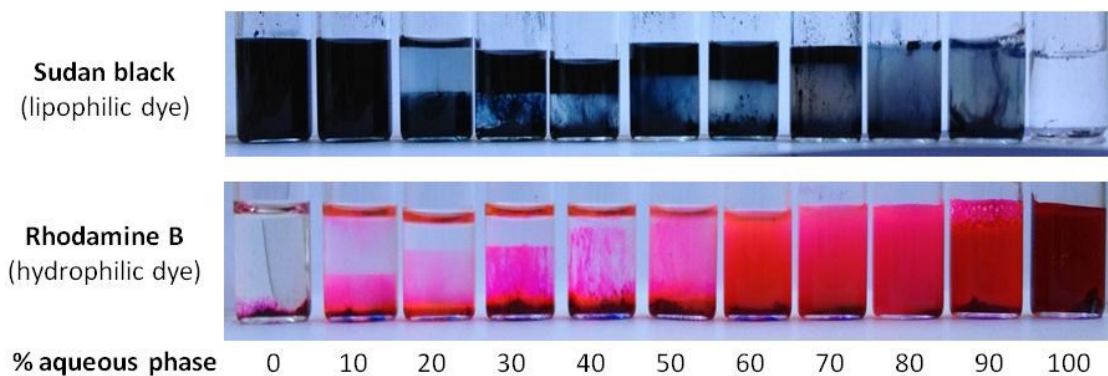
Figure S2: Samples prepared with an O/S = 70/30, at different percentages of aqueous phase (PBS = 0.16M), in the presence of a lipophilic and a hydrophilic dyes, after 5 min of dye addition.

The lipophilic dye diffused only at the lowest water contents, thus indicating a lipophilic continuous phase (e.g. W/O structures). In contrast, at high water contests, only the hydrophilic dye diffuses, indicating the formation of hydrophilic continuous phases (e.g. O/W structures). At intermediate water percentages, samples are very instable and destabilize rapidly, thus resulting in a diffusion of the lipophilic dye through the upper lipophilic phase and a diffusion of the hydrophilic dye through the lower hydrophilic phase. The upper phase corresponds to the lipophilic phase because the density of the ethyl acetate (lipophilic phase) is lower than that of the water.
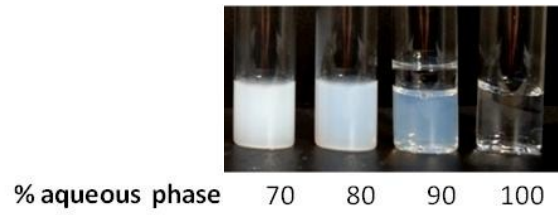
% aqueous phase   70   80   90   100

Figure S3: Examples of the visual appearance of nano-emulsions formulated with 70/30 O/S ratio, and different water phase percentages, at an electrolyte concentration of 0.16M.

The highest the water phase percentage, the more transparent and less turbid the visual appearance of the dispersions.
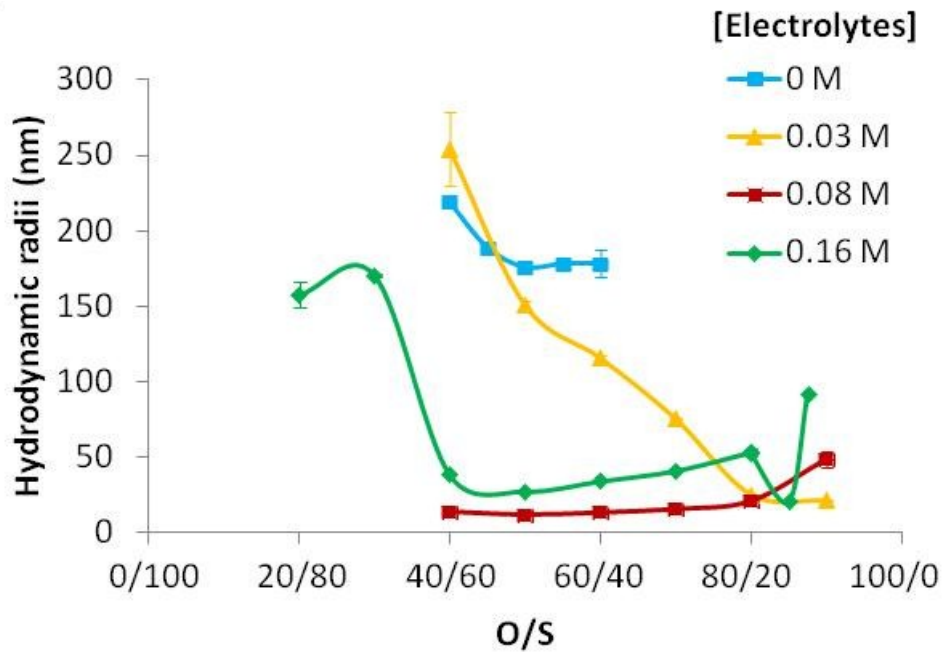
Figure S4: Hydrodynamic droplet radius of nano-emulsions prepared with 90wt % of aqueous phase, as a function of O/S ratio and electrolyte concentration.

This Figure shows the effect of the O/S ratio in the nano-emulsion droplet size, at the different electrolyte concentrations tested. At low electrolyte concentrations, at 0M, a size decrease from radii higher than 200 nm to radii of around 150 nm was found. The addition of electrolytes up to 0.03M produced a marked droplet size decrease when the O/S ratio was high. 0.08M electrolyte concentration resulted in the smallest droplet sizes, around 20 nm. The addition of more electrolytes up to 0.16M maintained small droplet sizes at O/S ratios higher than 40/60, but slightly higher than at 0.08M (around 30 nm), which could be caused by a coagulation effect due to this high electrolyte concentration.
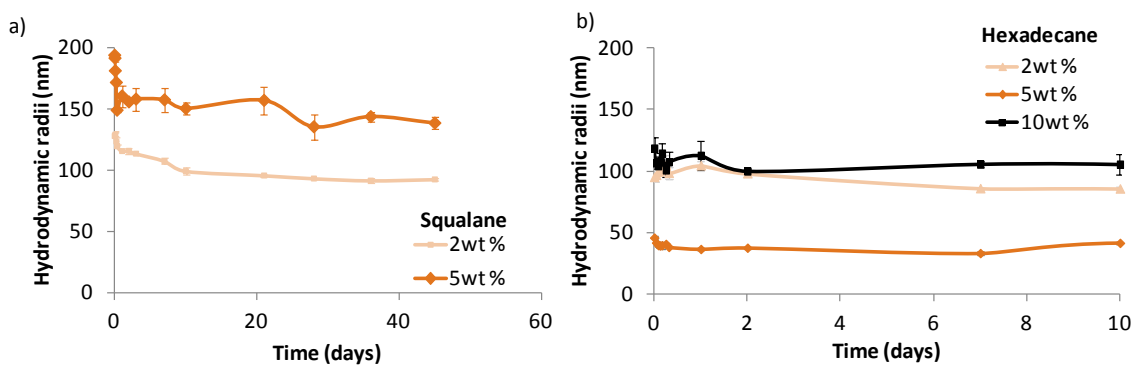
Figure S5: Hydrodynamic radii of nano-emulsions (90wt% of PBS 0.16M, 70/30 O/S), as a function of time and a) squalane and b) hexadecane concentration in the oil phase at 25ºC.

This Figure shows the evolution of droplet size of nano-emulsions with time. Since the addition of long hydrocarbon chains did not prevent nano-emulsion size fluctuations at shorter times, a destabilization caused mainly by Ostwald ripening can be ruled out.

Table S1: Refractive indexes of aqueous phases used for nano-emulsion preparation.

Electrolyte concentration (M)	Refractive index
0M	1.3330
0.03M	1.3335
0.08M	1.3346
0.16M	1.3351

The increase on the electrolyte concentration produced a slight increase in the refractive index.

Table S2: Solubility (in wt%) of the aqueous phase in the oil phase and of the oil phase in the aqueous phase assessed by visual observation. Results are expressed as means ± standard deviations of 10 replicates.

Samples tested	Solubility (wt%)
Water in ethyl acetate	3.0437 ± 0.0997
Electrolyte solution (0.16M) in ethyl acetate	2.4373 ± 0.1902
Ethyl acetate in water	7.2897 ± 0.1794
Ethyl acetate in electrolyte solution (0.16M)	6.7261 ± 0.0686

Explanation S1

The osmotic pressure can be calculated by the Van't Hoff equation (Equation S1) (Barnes, 2005):

$$\pi = iCRT \qquad\qquad (S1)$$

where i is the number of ions produced during the dissociation of the osmotic agents (electrolytes of the PBS salts in this case); C is the concentration of the osmotic agents (in M); R is the gas constant (0.08206 L·atm / mol·K) and T is the temperature (in K). Since R and T are constants, the osmotic pressure will depend only on the number and concentration of osmotic agents[40].

Table S3: Osmotic pressure of the four dispersant media used, with increasing electrolyte concentrations, calculated from Equation (S1).

Electrolyte concentration (M)	Osmotic pressure, Π (atm)
0M	0
0.03M	1.41
0.08M	3.52
0.16M	7.04

For nano-emulsions without electrolytes, the osmotic pressure will approach null values, due to the inexistence of osmotic agents. However, the incorporation of electrolytes increases the concentration of osmotic agents, thus producing an increase on the osmotic pressure.

When electrolytes are incorporated in the media, the number of dissociated molecules is the same, independently of the electrolyte concentration. Since

NaCl is the main electrolyte of the solution, it will be dissociated mostly in Na+ and Cl-, which are two ions, but some part of the molecules will remain as NaCl, thus having a number of dissociated molecules of 1.8.

The concentration of the ions of each electrolyte concentrations is their molarity, since the molarity is completely given by ionic species.

The calculated osmotic pressures correspond to the dispersant medium of nano-emulsions. Since inside the droplets no ionic species exist, the gradient of the osmotic pressure corresponds to those values of the calculated osmotic pressure of the dispersant medium.