# Low-energy nano-emulsifications: overview and potentials in microencapsulation

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## INTRODUCTION

Nanoparticles, defined as polymeric or lipid particles smaller than 300 nm, are of increasing interest in nano-medicine and nano-pharmaceutics today. They are considered as a tool to help open up a new dimension of treatments, penetrating cells and tissues, targeting diseased zones. However, despite their great potential, nanoparticles are as yet little known and controlled. One way of channeling this potential may be to generate nano-objects with very controlled surface and morphological properties in order to mimic "ideal" natural examples (e.g. viruses), along with a significant loading in active molecules. This would be intimately related to the control and understanding of their generating processes. Nanoparticles are mainly formulated through the generation of nano-emulsions (Anton N. 2008) which serve as templates for polymerization, nano-precipitation and lipid nano-crystallization. It therefore follows that understanding the mechanisms of nano-emulsification is of prime interest in all of these processes. Hence the interest of the current study which presents a new point of view on nano-emulsification, discloses a general and universal mechanism of nano-droplet generation.

Nano-emulsions consist of a mixture of immiscible liquids, where one of the liquids is dispersed in the form of nanometric-scaled droplets (20–300 nm) into the other one. The generating processes for nano-emulsions are divided into (i) high-energy and (ii) low-energy methods (Anton N. 2008). High-energy methods involve the use of specific devices such as high-pressure homogenizers or sonifiers, and only a very low amount (~0.1%) of the mechanical energy produced is used for emulsification (Tadros T.F. 2004). Low-energy methods divert the intrinsic physicochemical properties of the surfactants, co-surfactants and excipients in the formulation, leading to the generation of emulsion droplets in the nanometric range. Owing to the real advantages of low-energy methods in terms of formulation yields, potential industrial scale-up and non-aggressive features (e.g. against encapsulated fragile active molecules), there has been a real research interest in the development of such methods and techniques over the last twenty years. Numerous works lead to "classifying" the low-energy methods into very distinct emulsification procedures, *i.e.* distinct in the protocols (which depend on the nature of the excipients), but also distinct in the proposed mechanisms for the formation of nano-emulsion droplets. The two commonly reported low-energy nano-emulsification methods are (Anton N. 2008) the spontaneous emulsification method and the phase inversion temperature method (PIT method). In recent works (Anton N. 2009), we have shown that all these "different" low-energy nano-emulsification methods actually follows one unique and universal mechanism, based on the affinities of the surfactants molecules with the two immiscible phases. This new point of view generalizes the low-energy nano-emulsification methods, and clarifies the own understanding of the nano-emulsification mechanisms. These finding not only open doors to new alternative formulations which get round the use of toxic solvent or drastic formulation process, but also allow an easily adaptation of the process to the molecule encapsulation.





The present study takes benefit of these new ideas (i) to study first the formulation of fragile model molecule, *e.g.* vitamin E acetate, in the form of nano-emulsions, and (ii) second, to work out the own encapsulation of nano-emulsions into microparticles generated by classical spray-drying methods. Such a process aims to create multi-scaled objects, that are, hydro-soluble microparticles encapsulating nano-emulsions with high content of sensitive molecules, in order to allow their conservation, protection against degradation, but also to allow the "nano-redispersion" of nano-emulsions in the presence of water. The *nanometric scale* of nano-emulsions, is as well aimed to be conserved after redispersion, for improving (and keeping) the bioavailability which inheres in these nano-systems. Thus, all these points will be studied and evaluated experimentally, by dynamic light scattering, scanning electron microscopy, and HPLC.

## MATERIAL AND METHODS

### Material

Nonionic surfactant, Cremophor ELP® form BASF® was kindly furnished by Laserson (Etampes, France). It is parenteral-grade amphiphiles, PEG-35 Castor oil with HLB = 12-14. Vitamin E acetate was provided by Sigma (St. Louis, USA) and used as received. The different wall materials used for spray-drying are: Arabic gum from Colloïdes Naturels International (Rouen, France), Whey Protein form Davisco Foods international Inc. (USA), and Cleargum CO 03 (modified starch) and Glucidex IT 12 (maltodextrine, DE 12) were both kindly obtained from (Roquette, France). Ultrapure® water was produced from Millipore filtration system (Molsheim, France).

### Methods

***The generation of nano-emulsions by low-energy methods.*** Nano-emulsions are generated spontaneously by a universal low-energy method, without use of solvent, at room temperature, and within a few seconds. The protocol is described elsewhere (Anton N. 2009) and is governed by judicious choice of excipients, for which the amphiphiles have specific interactions with water and oil. Briefly, the process is based on a brutal displacement of the surfactant from the oily towards the aqueous phase. Cremophor ELP® is homogenized within the oily phase, and the mixing of this mixture with pure water gives rise to the immediate formation of nanometric-scaled emulsion droplets. Thus, a transposition of this process has been worked out with vitamin E acetate as oily phase. This "energy-free" and "solvent-free" process will appear particularly interesting since it insures the integrity of such a model of fragile molecule.

***Dynamic light scattering.*** Hydrodynamic diameters were obtained by dynamic light scattering using a Malvern NanoZS instrument. The helium–neon laser, 4mW, operates at 633 nm, with the scatter angle fixed at 173° and the temperature maintained at 25°C. Polydispersity index (PDI) is a mathematical definition that accounts for the relative error between curve fit and experimental values (Anton N. 2007). It shows the quality of the dispersion. Values $\leq 0.1$ reflect a very good monodispersity and quality of the nanoparticulate suspensions. Measurements were performed three times for each point.

***Spray drying.*** A Büchi Mini Spray-dryer B-290 (Flawil, Switzerland) apparatus is used for the formulation of microparticles. The two-fluid nozzle, operated by compressed nitrogen, disperses the solution into fine droplets, which are dried into hot air flow. The particles are then separated into a cyclone.

***Scanning electronic microscopy.*** Powders are observed with a scanning electron microscope Philips XL20, operating at 20 kV.

## RESULTS AND DISCUSSION

This section will present the main results of this study, that is, regarding the own formulation of vitamin E acetate nano-emulsions in comparison with our previous published results using model oils, characterized by DLS, in order to chose a compromise between droplet size, polydispersity and surfactant amount. Next, spray-dried microparticles are generated first with pure "wall material" and then loaded with vitamin E acetate nano-emulsions. The influence of the nature of the wall material, its concentration, and the {vitamin E acetate} / {wall material} weight ratio, on the particle size, polydispersity and morphologies, were investigated.

### Vitamin E acetate nano-emulsions

The optimum formulation of vitamin E acetate nano-emulsions was determined, by studying the impact of the surfactant / vitamin E acetate weight ratio, defined as SOR = 100 × $\{w_{surf.}\}$ / $\{w_{surf.} + w_{oil}\}$, $w_{surf.}$ and $w_{oil}$ being respectively the surfactant weight and the vitamin E acetate weight. The results, compared with the one of our previous works (ref. Anton N. 2009) are presented in Fig. 1 below. Hydrodynamic diameter and PDI are plotted against the SOR values.

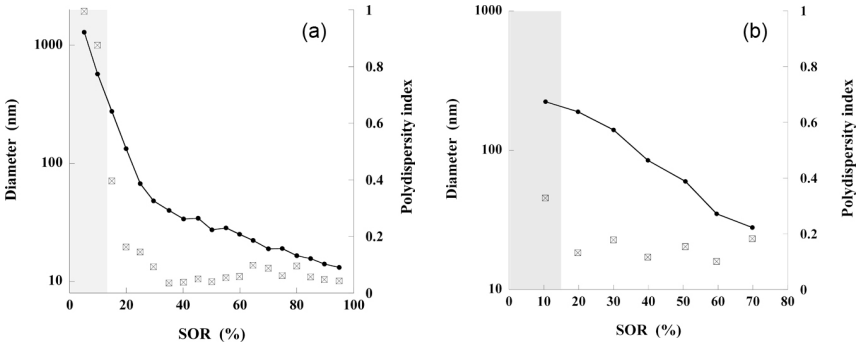


**Figure 1: A comparison between nano-emulsions formed by low-energy method. Hydro-dynamic diameter and PDI are plotted against SOR. (a) *Classical* formulation with oily systems from (Anton N. 2009), and (b) *transposition* of this formulation with substituting oil for pure active ingredient, vitamin E acetate.**

Briefly, the new results of Fig. 1 (b) appear coherent with the one of (a), lowering both the size and PDI when the surfactant amount is increased. That is a remarkable result, and show that this choice of compounds is compatible with the low-energy nano-emulsification. We choose for this conceptual study to focus on the first size below the barrier of 100 nm, *i.e.* for SOR = 40%. It also means that the encapsulation yield of vitamin E acetate is 100% and encapsulation rate is close to 100%.

### Microparticles formulation and characterization

The wall materials used for the nano-emulsion encapsulation were chosen from different natures, but classically used in spray-drying, *i.e.* Arabic gum, whey protein, modified starch and maltodextrine. Figure 2 shows, through some examples of SEM pictures with Arabic gum and whey protein, the morphology of microparticles formulated without and with nano-emulsions loading, at





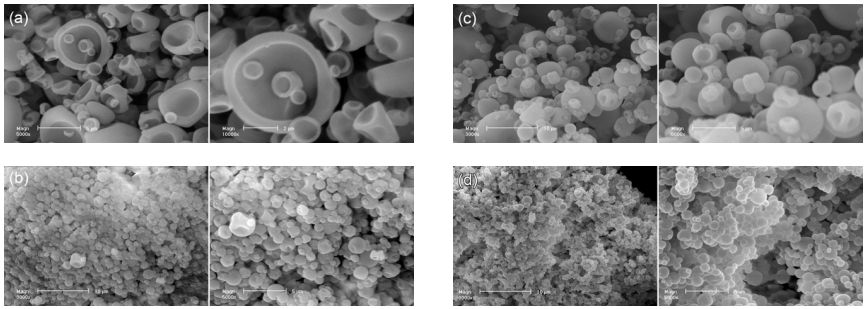


**Figure 2: SEM micrographs showing spray-dried microparticles. Concentrations of wall material are fixed at 15% for each sample. (a) Pure Arabic gum particles; (b) Arabic gum particles loaded with nano-emulsions; (c) Pure whey protein; (d) Whey protein particles loaded with nano-emulsions.**

the given wall material concentration of 15%, and (for nano-emulsion loaded particles) with SOR = 40%.

These results show that homogeneous particles encapsulating nano-emulsions can easily be produced by spray-drying, even improving their monodispersity and reducing their sizes. The nature of wall material clearly influences the particles morphologies and sizes. Such results showing the efficient encapsulation of nano-emulsions are new and important, since the difference of the order of magnitude between encapsulated forms (nano-emulsions) and the microparticulate final forms (microparticles), will allow improving the vitamin homogenous encapsulation, the vitamin retention, protection and conservation, along with a process which will not affect the structure of such a fragile molecule. Furthermore, HPLC quantification (not further detailed) shows that mirco-encapsulated vitamin E acetate (*after* spray-drying process) is no damaged by the formulation. In addition, stability studies show that the integrity of vitamin E acetate is conserved over a few months, whereas it is rapidly altered when it is stocked in solution. Lastly, the dried microparticles are shown to "re-solubilize" in water in a few seconds (related to high specific surface), and still releasing nano-emulsions suspensions.

## CONCLUSION

With this study, we propose some new ideas to encapsulate sensitive molecules within nano-emulsions themselves encapsulated in hydrosoluble microparticles, in order to improve their protection, conservation, but also keeping unchanged the properties which inhere in nano-emulsions after redispersions in water. As well, that could allow improving administration and bioavailability of such fragile principles.

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