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Preparation of vitamin E loaded nanocapsules by the nanoprecipitation method: From laboratory scale to large scale using a membrane contactor

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ABSTRACT

Vitamin E or α-tocopherol is widely used as a strong antioxidant in many medical and cosmetic applications, but is rapidly degraded, because of its light, heat and oxygen sensitivity. In this study, we applied the nanoprecipitation method to prepare vitamin E-loaded nanocapsules, at laboratory-scale and pilot-scale. We scaled-up the preparation of nanocapsule with the membrane contactor technique. The effect of several formulation variables on the vitamin E-loaded nanocapsules properties (mean diameter, zeta potential, and drug entrapment efficiency) was investigated. The optimized formulation at laboratory-scale and pilot-scale lead to the preparation of vitamin E-loaded nanocapsules with mean diameter of 165 and 172 nm, respectively, and a high encapsulation efficiency (98% and 97%, respectively).

1. Introduction

Vitamin E is a lipid-soluble antioxidant often regarded to α-tocopherol (Ne Cheong et al., 2008). α-Tocopherol is one of many natural antioxidants widely used in vitamin supplementation, food, cosmetic and pharmaceutical industries (Constantinides et al., 2006). Most of these antioxidants are almost insoluble in water or show very low water solubility. This poor water solubility has made their use problematic in food formulation (Tan and Nakajima, 2005), and in classic pharmaceutical forms, leading to insufficient bioavailability especially after oral or parenteral/intravenous administration and very often below the therapeutic level (Muller and Peters, 1998).

Nanoparticles represent very promising drug-delivery systems. Nanoparticles regroup both nanocapsules and nanospheres. According to the literature, the nanocapsules correspond to a polymeric wall enveloping an oil core, whereas the nanospheres consist of polymeric matrix (Megenheim and Benita, 1991). One of the advantages of nanocapsules over nanospheres is their low polymeric content and high loading capacity for lipophilic drugs (Legrand et al., 1999). Other advantages of confining the drug within a central cavity is that the burst effect may be avoided, the drug is not in direct contact with tissues and therefore irritation at the site of administration will be reduced, and that the drug may be better protected from degradation both during storage and after administration (Couvreur et al., 2002). This property is of high interest for vitamin E encapsulation because of its high sensitivity to light, heat and oxygen (Bouchemal et al., 2003). Besides, the problem of solubility of vitamin E in water can be avoided as the final form is an aqueous suspension of vitamin E-loaded nanocapsules.

The objectives of our work were firstly to optimize the formulation of vitamin E-loaded nanocapsules in terms of mean diameter, zeta potential, and secondly to scale up this formulation at pilot scale by increasing 8-folds the laboratory batch volume from 75 ml to 600 ml. The pilot scale preparations were obtained using a membrane contactor technique and the effect of process variables (organic phase pressure, aqueous phase flow rate and mean membrane pore diameter) were investigated on the characteristics of the produced nanocapsules.

For all vitamin E-loaded nanocapsule preparations, the nanoprecipitation method was used. The nanoprecipitation method is an easy and reproducible method involving dispersion of preformed polymers (Fessi et al., 1989), based on the interfacial deposition of polymer following displacement of semi-polar solvent miscible

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with water from lipophilic solution. The organic phase (solvent, polymer, eventually oil, and drug) is added drop wise under moderate stirring into the aqueous phase (water, and surfactant).

The membrane contactor technique has been previously applied to the preparation of emulsions ("membrane emulsification") (Charcosset et al., 2004; Charcosset and Fessi, 2009). It has also been reported for the preparation of polymeric nanospheres and nanocapsules (Charcosset and Fessi, 2005; Limayem Blouza et al., 2006), lipid nanoparticles (Li et al., 2011; D'Oria et al., 2009), and liposomes (Jaafar-Maalej et al., 2011; Laouini et al., 2011). In our previous study (Charcosset and Fessi, 2005), polymeric nanospheres and nanocapsules have been prepared using a ceramic membrane, classically used in filtration, with a high flux through the membrane and large membrane pore size distribution. The technique has been applied to the loading of sprinolactone in nanoparticles (Limayem Blouza et al., 2006). In the present study, we prepare polymeric nanoparticles with a Shirasu porous glass (SPG) membrane, which is specifically designed for membrane emulsification and therefore presents a much sharper pore size distribution than classical ceramic membranes. The technique is applied to the preparation of vitamin E loaded nanocapsules.

2. Materials and methods

2.1. Materials

Vitamin E was supplied by Sigma–Aldrich Chemicals (France).

Polycaprolactone (PCL) with molecular weight of 10 000 and poly(DL-lactide-co-glycolide) (PLGA) with molecular weight 50,000–75,000 (lactide:glycolide (85:15) were supplied by Sigma–Aldrich Chemicals (France).

The surfactants Tween® 80 and Tween® 20 were purchased from Prolabo (France), Poloxamer®407 from Sigma–Aldrich Chemicals (France), Lipoid® E 80 was obtained from Lipoid GMBH (Ludwigshafen, Germany) and sodium Lauryl sulfate from Sigma–Aldrich (France). All these products were analytical grade pure.

The oils (castor oil, sesame oil) were obtained from Crystal (China), and Labrafac Hydro® was obtained from Gattefosse (France).

The oils were pharmaceutical grade. PCL and PLGA are biodegradable polymers used for pharmaceutical preparations and have no reported toxicity.

Analytical grade acetone was provided from Surechem Products LTD (England). HPLC grade methanol and acetonitril were provided from Merck (Germany).

2.2. Preparation of vitamin E-loaded nanocapsules at laboratory-scale

The vitamin E-loaded nanocapsules were prepared using the nanoprecipitation method first developed by Fessi et al. (1988, 1989). PCL and vitamin E were dissolved in acetone at 30 °C using a water bath. The organic solution was then added drop wise, using a syringe, at the rate of 5 ml min^{-1} into the aqueous phase containing the hydrophilic surfactant under moderate magnetic stirring, at 25 °C.

The aqueous phase immediately turned milky with bluish opalescence due to the formation of the nanocapsule suspension. The acetone was then evaporated at 40 °C under reduced pressure, using a rotavapor (Rotavapor R-144, Buchi, Flawil, Switzerland) for approximately 30 min. Finally, the nanocapsule suspension was concentrated to final volume of 35 ml by removal of water under the same conditions.

Every preparation was repeated at least three times.

The optimization of the formulation in each step was determined by measuring the mean size, polydispersity index, zeta potential, observing the absence or presence of aggregates, and in some cases, by determination of the entrapment efficiency (Table 1).

2.2.1. Optimization of oil

The criteria for selecting the best oil are low toxicity, low solubility of the polymer in the oil and the absence of degradation of the polymer (Couvreur et al., 2002).

Besides, in case of a lipophilic active substance, it should be dissolved in oil chosen for its highest possible drug solubility in order to form nanocapsules and not nanospheres. It is emphasized that different capric/caprilic triglyceride types are often used because of their wide range of solubility for active substances. Although other oils such as sunflower seed oil and soybean oil have not been frequently used, they can nonetheless give good results (Mora-Huertas et al., 2010).

Three types of oils were tested: castor oil, sesame oil, and Labrafac Hydro®. Nanocapsules were prepared with 0.5 ml of each of the oils separately. All other constituents of the formulation were unchanged: 125 mg PCL, 100 mg α-tocopherol, 50 mg Tween 80, 25 ml acetone, and 50 ml water. The fourth formulation was prepared without adding oil to the α-tocopherol solution.

2.2.2. Determination of polymer quantity and type effect

Vitamin E-loaded nanocapsules were prepared with different amounts of PCL: 50, 100, 125 and 200 mg, respectively.

Another polymer was replaced only for comparison, PLGA with molecular weight between 50,000 and 75,000 (lactide:glycolide) (85:15). All other constituents of the formulation were unchanged.

2.2.3. Optimization of surfactant type and quantity

The effect of the surfactant amount was studied with Tween® 80. The different amounts of Tween® 80 were (25, 50, 100 mg).

The effect of surfactant type was investigated with Tween® 80, poloxamer® 407, sodium Lauryl sulfate (SDS) and a mixture of Tween 80 with lipophilic surfactants (Lipoid® E 80 or Tween® 20).

2.2.4. Determination of vitamin E amount effect

Three amounts of vitamin E were tested (50, 100, 150 mg), all other constituents were unchanged.

The effect of vitamin E quantity was evaluated by measuring the mean size, polydispersity index, zeta potential, and the entrapment efficiency.

2.2.5. Determination of the adding order effect

The effect of adding either the organic phase into the aqueous phase or the aqueous phase into the organic phase was investigated by comparing the same optimized formulation prepared with different addition order and by using same adding rate (5 ml min^{-1}).

2.3. Preparation of vitamin E-loaded nanocapsules at pilot-scale using a membrane contactor

2.3.1. Experimental set-up

The experimental set-up used for preparation of nanocapsules by the membrane contactor technique is shown in Fig. 1. It included a pump (Filtron, Germany), a nitrogen bottle, and a pressurized recipient (Millipore) with a manometer M1, a metallic device with the cylindrical SPG membrane inside (SPG Technology, Japan), and two manometers M at the inlet and outlet of the device.

Tubular SPG membranes were purchased from SPG Technology (Miyazaki, Japan). SPG membranes are prepared by phase-separated glass leaching in the

Table 1
Variable constituents used to optimize the formulation for the preparation of vitamin E-loaded NCs.

	Constituents		Quantity			
Organic phase	Solvent (ml)	Acetone	25			
	Polymer (mg)	PCL	50	100	125	200
		PLGA			125	
	Active ingredient (mg)	α-tocopherol	50	100	150	
	Oil (ml)	Castor oil	0.5			
		Sesame oil	0.5			
		Labrafac Hydro®	0.5			
	Hydrophobic surfactant (mg)	Lipoid® E 80	40			
		Tween® 20	50			
Aqueous phase	Non-solvent (ml)	Water	50			
	Hydrophilic surfactant (mg)	Tween® 80	50			
		Poloxamer® 407	50			

Na_2–O–CaO–MgO–Al_2O_3–B_2O_3–SiO_2 system, which is synthesized from volcanic ash, called Shirasu, used as the main raw material (Vladisavljević et al., 2005, 2007). SPG membranes have been extensively used for the preparation of emulsions and particulate products ranging from simple o/w and w/o emulsions, different multiple emulsion types, solid lipid microspheres, polymeric microspheres, core–shell microcapsules, and hollow polymeric micro particles (Vladisavljević and Williams, 2005). In the present study, three membranes with different pore size were investigated: 0.2, 0.9, and 10.2 μm hydrophilic SPG membranes.

The SPG membrane dimensions were as follows: 0.125 m in length, 10^{-2} m in inner diameter, and 10^{-3} m in thickness. Hence, the active membrane surface was 3.9×10^{-3} m^2.

2.3.2. Nanocapsules preparation using a membrane contactor

The following protocol was used for experiments, and was the same as described previously (Charcosset and Fessi, 2005). The aqueous phase was stirred continuously using a magnetic stirrer and circulated tangentially to the membrane surface. The organic phase was placed in the pressurized vessel, connected to nitrogen bottle and to the membrane module on the filtrate side. At time $t = 0$, the valves connecting the pressurized vessel to the nitrogen bottle and to the filtrate side of the membrane module were opened. The aqueous phase immediately turned milky with bluish opalescence as a result of the formation of nanoparticles. The experiment was stopped when air bubbles started to appear in the tube connecting dispersed vessel to the membrane module. The experiments were conducted at 22 ± 1 °C. The preparation obtained was then evaporated under reduced pressure at 40 °C (Rotavapor R-144, Buchi, Flawil, Switzerland) to eliminate the solvent and to concentrate the nanocapsule suspension. At the end of the experiment, a sample of nanoparticles preparation was taken for size analysis.

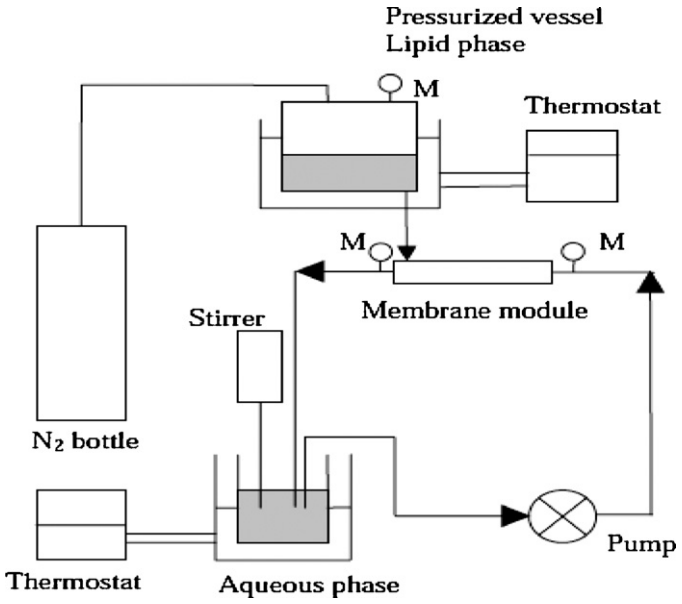

Fig. 1. Experimental set-up for the preparation of nanoparticles. M: manometer.

The microfiltration membrane was then regenerated. The washing was performed by flushing the membrane module with mixture of 0.2 l acetone with 0.4 l of pure water in an open cycle, then with 300 ml acetone in the pressurized vessel (0.5 bar) and 1 l of water circulating tangentially to the membrane surface, and finally with 3 l pure water circulated in a closed cycle for 30 min. To complete the cleaning, the membrane was placed in an ultrasound bath (Bandelin, France) for 15 min in 30 ml of a mixture of acetone:water (35:65).

The membrane permeability was measured at the beginning of each experiment and was checked to be around 90% of its initial value. The reproducibility of both laboratory-scale and pilot-scale preparations were estimated on three experiments, performed at the same operating conditions.

The selected formulation obtained from the laboratory-scale study: (125 mg PCL, 25 ml acetone, 100 mg vitamin E, 50 mg Tween® 80, 50 mg water) was scaled-up for the membrane contactor 8-folds.

2.3.3. Determination of organic phase pressure effect

Various dispersed phase pressures were used for the preparations (0.2, 0.5, 1, 1.5 bar). Experiments were performed using the 0.9 μm SPG membrane and the optimized formulation (1000 mg PCL, 800 mg vitamin E, 200 ml acetone, and 400 mg Tween 80, and 400 ml water). The aqueous phase flow rate was maintained equal to 700 ml min^{-1}.

2.3.4. Determination of aqueous phase flow rate effect

The effect of this parameter on the mean particle size, polydispersity index, zeta potential of prepared vitamin E-loaded nanocapsules and the absence/presence of aggregates was investigated. The flow rate of the circulating aqueous phase was controlled by the pump. For these experiments, the dispersed phase pressure was equal to 0.5 bar and the 0.9 μm SPG membrane was used. Three flow rates were tried (200, 700, 1000 ml min^{-1}).

2.3.5. Determination of mean membrane pore size effect

Three SPG membranes with different mean pore size were used (0.2, 0.9, 10.2 μm). The organic phase pressure and aqueous phase flow rate were kept constant (0.5 bar, 700 ml min^{-1}).

In case of the 0.2 μm mean pore size membrane, a minimum dispersed phase pressure was required for nanocapsule preparation. Below this limiting pressure (1 bar), the organic phase was not able to pass through the membrane pores.

2.4. Nanocapsules characterization

2.4.1. Size analysis

The mean size of the vitamin E-loaded nanocapsules, polydispersity index (PdI) and zeta potential was determined by photon correlation spectroscopy (PCS), using a Malvern Zetasizer Nano-series (Nano-ZS, Malvern Instruments, Malvern, UK). The PdI values range from 0 to 1; a higher value indicates a less homogeneous nanocapsule size distribution. Zeta potential was measured by Smoluchowski's equation from the electrophoretic mobility of nanocapsules. All measurements were performed at 25 °C.

2.4.2. Microscopic observation

Vitamin E-loaded nanocapsules were imaged using a transmission electron microscope (TEM) and scanning electron microscope (SEM). TEM pictures were taken with CM 120 microscope (Philips, France) operating at 80 kV acceleration. The sample preparation was realized according to similar previous studies (Guinebretière et al., 2002). The vitamin E-loaded nanocapsules were diluted by a factor of 30 and deposited on a carbon-coated copper grid. Negative staining with a 1% sodium phosphotungstate solution was made directly on the deposit.

Morphological examination of vitamin E-loaded nanocapsules was performed using a SEM (Hitachi S800 FEG microscope, Japan). A drop of diluted aqueous suspension of nanocapsule was deposited on a flat steel holder and dried at room temperature. The sample was finally coated under vacuum by cathode sputtering with palladium. The samples were observed by SEM under an accelerating voltage of 15 kV.

2.4.3. Encapsulation efficiency

Total vitamin E concentration (T_E) was determined after dissolution of 1 ml of vitamin E-loaded nanocapsules in 10 ml acetonitril.

Free vitamin E concentration (F_E) was determined after separation of loaded-nanocapsules from the aqueous medium by ultracentrifugation (CP 80WX Himac preparative ultracentrifuge, Hitachi, Japan). Samples were centrifuged at 45 000 rpm for 30 min at 20 °C. The free vitamin E concentration was then determined in the supernatant.

HPLC separation was then performed with Shimadzu Liquid chromatographer (LC-20AT, Shimadzu, Kyoto, Japan) equipped with SPD-M20A photodiode array detector, LC-20AT pump system, CTO-20A oven, and SIL-20A auto sampler. Quantitative measurement of a-tocopherol content was done at $\lambda = 285$ nm. The α-tocopherol was separated on a silica gel column (EC 150/4.5 Nucleodur 100-5 C18, Macherey-Nagel, Germany) with a mobile phase of methanol:water (99:1) (v/v) at 1.0 ml min^{-1}. The measurement was performed at 25 °C. The calibration of peak area versus α-tocopherol concentration was linear in the concentration range of (0.2–1.0 mg/ml) ($R^2 = 0.9998$). Injections, in triplicate, were done at each concentration for standards and samples. The analytical method was validated as usually required.

The encapsulation efficiency was calculated as follows:

$$\text{Encapsulation efficiency}\,(\%) = \frac{T_E - F_E}{T_E} \times 100 \quad (1)$$

According to the literature, nanoprecipitation is one of the methods which give best results for nanocapsule encapsulation (80% or more) (Mora-Huertas et al., 2010). Different factors determine the drug encapsulation efficiency such as the chemical nature of the drug and its polarity. In this sense, lipophilic compounds show high encapsulation efficiency. As vitamin E is oil, its EE could reach 99% (Ma et al., 2001; Stella et al., 2007). These high values of EE% can be a proof that PCL has no solubility in α-tocopherol.

3. Results and discussion

3.1. Preparation of vitamin E-loaded nanocapsules at laboratory-scale

3.1.1. Optimization of oil

For the development of vitamin E nanocapsule suspension, suitable oil needs to be chosen.

The effect of the oil type on nanocapsule mean size, polydispersity index, zeta potential and aggregation is presented in Table 2.

It is noticed that the nanoparticles prepared with castor oil were the largest, due to the higher viscosity of this oil. We assume that when the oil viscosity is higher, then the dispersed phase viscosity increases. In addition, the polydispersity index elevated when the oil viscosity increased. This result was similar to that reported by Pohlmann et al. (2002) who also noticed that lower viscosity cause decrease in the interface tension and as a consequence the prepared emulsion gave submicron particles with little population of micro particles in case of biodegradable polymers proportional increase in the particle diameter and the polydispersity index with an increase of oil viscosity.

The smallest particle size of vitamin E-loaded nanocapsules (165 nm) and polydispersity index (0.18) were obtained without adding any additional oil to the vitamin E solution. Zeta potential was negatively charged due to the presence of polymer terminal carboxylic groups, knowing that the stabilizer used (Tween® 80) is a non-ionic stabilizing agent. The lower value (−16 mV) was obtained for the preparation of nanocapsules without adding additional oil to the vitamin E solution. The literature reports that zeta potential values lower than −10 mV allow predicting good colloidal stability due to the high energy barrier between particles (Mora-Huertas et al., 2010).

It can be concluded that using oil inversely affected the size of NCs produced, and led to lower zeta potential values which might indicate less stable nanocapsules. In addition, avoiding the usage of an additional excipient means less toxic effect. On the other hand, this gives the opportunity for a wider range of applications, especially when regarding the parenteral use. In cases of hydrophobic substances, using oil is more necessary for solubility.

For these reasons, further studies were done without adding oil.

3.1.2. Determination of polymer quantity and type effect

The effect of PCL amount on the nanocapsule size, polydispersity index and zeta potential is presented in Table 3. The smallest particle size of vitamin E-loaded nanocapsules (150 nm) was obtained with the 50 mg amount of PCL. Little change in mean size, polydispersity index and zeta potential was observed when changing the polymer amount.

One assumption to explain this result is that the shell thickness is not necessarily the same when different amounts of PCL are used. According to different authors, the shell thickness values were about 10 nm (Rube et al., 2005) and 20 nm (Cauchetier et al., 2003) when PCL was selected as polymer by the nanoprecipitation method and 10 nm when PLGA was chosen (Nassar et al., 2009). It has been reported that a higher polymer concentration in the oil phase led to an increase in shell thickness of the nanocapsules obtained (Cauchetier et al., 2003). This means, when duplicating the polymer quantity from 50 to 100 mg, the number of produced NCs will not be necessarily doubled.

From 200 mg PCL, the nanoparticle size increased affecting the zeta potential value. The increase in droplet size means decreasing the total surface area of the nanocapsules. The surface of the nanocapsules is negatively charged due to carboxylic terminals of the polymer as previously mentioned. The increase in the zeta potential value may be due to the negative charged surface area

Table 2
Effect of the oil type on the mean size of vitamin E-loaded nanocapsules, polydispersity index, zeta potential and presence of aggregates.

Sample	Type of oil	Dynamic viscosity at 20 °C mPas (cP)	Mean diameter (nm) ± SD[a]	PdI ± SD[a]	Zeta potential ± SD[a] (mV)	Aggregates
NC_1	Castor oil	1000	320 ± 26	0.50 ± 0.09	−9.58 ± 2.3	++
NC_2	Sesame oil	43	311 ± 21	0.41 ± 0.12	−1.46 ± 0.75	+
NC_3	Labrafac Hydro®	27–30	247 ± 15	0.39 ± 0.19	−9.57 ± 5	–
NC_4	–	–	165 ± 16	0.18 ± 0.008	−16 ± 3	–

The viscosity data are taken from Rowe et al. (2009).
[a] $n = 3$; SD: standard deviation between the three assays.

Table 3
Effect of polymer amount and type on the mean size of vitamin E-loaded nanocapsules, polydispersity index, zeta potential and presence of aggregates.

Sample	Polymer type	Polymer amount (mg)	Mean diameter (nm) ± SD[a]	PdI ± SD	Zeta potential ± SD[a] (mV)	Aggregates	EE%
NC_5	PCL	50	150 ± 7	0.23 ± 0.03	−18.4 ± 0.85	–	96.5 ± 0.16
NC_6		100	154 ± 10	0.25 ± 0.07	−12.3 ± 0.8	–	97.7 ± 0.06
NC_7		125	165 ± 16	0.23 ± 0.08	−16.1 ± 3.77	–	98 ± 0.4
NC_8		200	195 ± 8	0.24 ± 0.03	−5.15 ± 3.25	–	ND
NC_9	PLGA	125	132 ± 12	0.07 ± 0.02	−5.24 ± 1.69	–	ND

ND: Not determined.
[a] $n = 3$; SD: standard deviation between the three assays.

which became smaller and/or resulting from more coverage of non-ionic stabilizing agent of the negative charge.

It is noticed that the nanocapsule mean size was a little smaller with PLGA than with PCL. This result was similar to that when nanoparticles loaded with a pure antiestrogen was investigated by Ameller et al. (2003). The polydispersity index was noticeably lower in case of nanocapsules prepared with PLGA. The zeta potential increased when PCL was replaced by PLGA; this could be due to the chemical structure of the polymer. PLGA has less free carboxylic terminals deactivated by the esteric bond between the two carboxylic acids resulting in slightly higher value of zeta potential.

Lemoine et al. (1996) have found that the stability of polymeric nanoparticles depended on the type of polymer with the following increasing order: PLGA < PLA = PCL. This could be predictable when comparing the zeta potential values resulting from PCL NCs and PLGA NCs. In addition, the PCL quantity had little effect on encapsulation efficiency, but 125 mg of PCL gave the highest EE%. For these reasons, PCL was chosen for the following experiments.

3.1.3. Optimization of surfactant type and quantity

With the surfactant Tween® 80, the smallest particle size of vitamin E-loaded nanocapsules was 165 nm (Table 4).

From the 50 mg data, an increase or decrease in the surfactant amount produced an increase in nanocapsule mean size. It can be concluded that a lower amount of stabilizer than 50 mg was not sufficient to prevent coalescence during the nanocapsule formation. Adding more surfactant led to more stable nanocapsule with less coalescence and lower mean size, as shown in Table 4. Above this value, the mean diameter of the nanocapsules increased again.

It can also be noticed that the surfactant type played a role in changing the mean size of loaded-vitamin E nanocapsules (Table 4). This result is very similar to that reported by Limayem Blouza et al. (2006) who observed that among all surfactants tested, the Tween® 80 gave the smallest particle size.

Zeta potential values were nearly the same for of all non-ionic surfactants. This may be explained by the similar nanocapsule surface produced in the different cases. When the ionic surfactant negatively charged (SDS) was used, an important lowering in zeta potential value was noticed. In addition, the ionic nature of this surfactant may cause problems of chemical stability of the NCs and less safety to the human body. SDS is a moderately toxic material with acute toxic effects including irritation of the skin and the mucous membranes and it should not be used in intravenous preparations for humans. This could limit the applications of this preparation, which could be helpful in oral topical or parenteral use.

Hence, 50 mg of Tween® 80 was retained for further experiments.

3.1.4. Determination of vitamin E amount effect

The effect of vitamin E amount on the nanocapsule size, polydispersity index, zeta potential value and entrapment efficiency is shown in Table 5. The smallest particle size of vitamin E-loaded nanocapsules (143 nm) was obtained when 50 mg of vitamin E was used.

The nanocapsule mean size increased with the increase of vitamin E amount. This result may be explained by the high viscosity of the α-tocopherol oil. On the other hand, the zeta potential decreased with the increase of the oleic amount. This increase in zeta potential value is expected to occur simultaneously to the increase of particle diameter as previously explained. The encapsulation efficiency of vitamin E was high due to the high lipophilicity of vitamin E. The encapsulation efficiency is usually proportional to the lipophilicity of the drug to be encapsulated. It has been reported that in cases of lipophilic drugs encapsulation efficiency higher than 70% was obtained (Ma et al., 2001; Stella et al., 2007). In addition, using different amounts of vitamin E in the preparation led to no significant change in encapsulation efficiency as shown in Table 5. Hence, 100 mg of vitamin E was retained for further studies.

3.1.5. Determination of the adding order effect

The effect of adding the aqueous phase into the organic phase versus adding the organic phase into the aqueous phase was determined. The vitamin E-loaded nanocapsules were prepared using the aqueous phase (50 ml water, 50 mg Tween® 80), and the organic phase (25 ml acetone, 100 mg vitamin E, 125 mg PCL). The results are presented in Table 6.

Obvious aggregation between particles was observed when the aqueous phase was added to the organic phase, this is because the stabilizer, located in the aqueous phase, plays an important role in stabilizing the nanocapsules formed. Hence the organic phase was added to the aqueous phase in all other experiments.

3.2. Preparation of vitamin E-loaded nanocapsules at pilot-scale using a membrane contactor

At laboratory-scale, the average mean size of the selected formulation of vitamin E-loaded nanocapsules (organic phase: PCL

Table 4
The effect of surfactant amount and type on mean size, polydispersity index and zeta potential of vitamin E-loaded nanocapsule.

Sample	Surfactant type	Surfactant Amount (mg)	Mean size (nm) ± SD[a]	PdI ± SD[a]	Zeta potential ± SD[a] (mV)
NC_{10}	Tween® 80	25	193 ± 15	0.25 ± 0.08	−19.7 ± 0.73
NC_7		50	165 ± 16	0.23 ± 0.08	−16.1 ± 3.77
NC_{11}		100	195 ± 14	0.27 ± 0.05	−16.6 ± 2.2
NC_{12}	Poloxamer® 407	50	203 ± 10	0.12 ± 0.025	−14.3 ± 0.28
NC_{13}	Lipoid® 80/Tween® 80	40/50	222 ± 23	0.72 ± 0.042	−24.5 ± 3.5
NC_{14}	SDS®	50	189 ± 8	0.091 ± 0.001	−61 ± 1.03
NC_{15}	Tween® 20/Tween® 80	50/50	190 ± 7	0.19 ± 0.0193	−12.3 ± 2.04

[a] $n = 3$; SD: standard deviation between the three assays.

Table 5
The effect of vitamin E amount on the mean size, polydispersity index, zeta potential, and entrapment efficiency of vitamin E-loaded nanocapsule.

Sample	Vitamin E (mg)	Mean diameter (nm) ± SD[a]	PdI ± SD[a]	Zeta potential (mV) ± SD[a]	EE% ± SD[a]
NC_{16}	50	143 ± 5	0.12 ± 0.013	−20.6 ± 1.3	97.7 ± 0.6
NC_7	100	165 ± 16	0.23 ± 0.08	−16.1 ± 3.77	98 ± 0.4
NC_{17}	150	236 ± 7	0.27 ± 0.06	−8.11 ± 0.81	96.7 ± 0.32

[a] $n = 3$; SD: standard deviation between the three assays.

Table 6
The adding order effect on the mean size, polydispersity index, zeta potential, and entrapment efficiency of vitamin E-loaded nanocapsule.

Sample	Adding order	Mean size (nm) ± SD[a]	PdI ± SD[a]	Zeta potential (mV) ± SD[a]	Aggregations
NC_7	Organic to aqueous	165 ± 16	0.23 ± 0.08	−16.1 ± 3.77	–
NC_{18}	Aqueous to organic	123 ± 20	0.64 ± 0.16	−19.8 ± 0.4	++

[a] $n = 3$; SD: standard deviation between the three assays.

125 mg, vitamin E 100 mg, acetone 25 ml, aqueous phase: Tween® 80 50 mg, water 50 ml) was found equal to 165 ± 16 nm. This formulation was scaled-up (8-folds) using the membrane contactor technique. The influence of three operating parameters was investigated: the organic phase pressure, the aqueous phase flow rate, and the mean membrane pore diameter. Only one parameter was changed at a time in the different experiments.

3.2.1. The effect of organic phase pressure

The effect of the organic phase pressure was investigated by trying different pressure values and maintaining the other experimental conditions (mean membrane pore size 0.9 μm, aqueous phase flow rate: 700 ml min^{-1}). It is obvious that the greater pressure of organic phase used, the greater mean size of vitamin E-loaded nanocapsule was obtained, with high aggregation (Table 7). This phenomena was also reported by Charcosset et al. (2004) who noticed that the average droplet size and size distribution tended to increase with the dispersed phase pressure because of increased droplet coalescence at the membrane surface. In case of low transmembrane pressure (0.2 bar), this result was explained by Kukizaki (2009) who assumed that the droplets remain attached to the membrane surface until they are pushed away by the next droplet formed at the pore opening when using non-anionic surfactant as Tween 80, as used in our study. This results in coalescence between the droplets during droplet formation from the membrane pores and consequently increased the mean diameter of nanocapsules formed. This explanation may be clearer when regarding the zeta potential values. The coalescence of droplet size decreased the total surface area of nanocapsules and increased the zeta potential value, because firstly the negative charged surface area became smaller and secondly the non-ionic stabilizing agent was perhaps covering a larger surface area of nanocapsules.

In addition, the preparation time decreased with the organic phase pressure as predicted by the classical Darcy's law through porous media. No aggregation was observed at 0.2 bar (preparation time 6 min) and at 0.5 bar (preparation time 4.5 min). In further experiments, the 0.5 pressure was selected because leading to the faster preparation with no aggregation.

3.2.2. The effect of aqueous phase flow rate

The effect of the aqueous phase flow rate on the mean size of vitamin E-loaded nanocapsules is shown in Table 8.

The droplets formed at the membrane surface detach under the influence of flowing continuous phase. The characteristic parameter of the flowing continuous phase is the flow rate, the cross flow velocity or the wall shear stress. The flow rate did not show an effect on the nanocapsule size diameter below 700 ml min^{-1}. When a flow rate of 1000 ml min^{-1} was used, the mean size of nanocapsules began to increase. This may be due to coalescence between the nanocapsules formed when reaching a high flow rate. Zeta potential values were almost the same for the different velocities. The preparation time did not change with the tangential flow value and was found around 4.1–4.5 min. This may indicate that low membrane fouling occurred on the membrane surface. Indeed, high fouling would be reduced by using higher tangential flow rates on the membrane surface, and in this case the preparation time would decrease.

3.2.3. The effect of mean membrane pore diameter

Three hydrophilic SPG membranes were used, with different mean pore size (0.2, 0.9, 10.2 μm). The mean size of vitamin E-loaded nanocapsules increased when the mean membrane pore size increased (Table 9).

In "membrane emulsification", it is recognized that the average of droplet diameter, $\bar{d}_d$ increases with the average membrane pore diameter, $\bar{d}_p$, by the linear relationship, for given operation conditions (Charcosset et al., 2004):

$$\bar{d}_d = c\bar{d}_p$$

where c is a constant.

In the present study on nanocapsule formation, the mean diameter of nanocapsules did not change when increasing the mean membrane pore size. We can say that in the first case (mean pore size 0.2 μm); the mean diameter of droplets formed was equal to the mean pore size. This size remained unchanged when increasing the membrane pore size. Zeta potential values did not change significantly, which correlated with the absence of effect of the

Table 7
The effect of organic pressure on the mean size, polydispersity index and zeta potential of vitamin E-loaded nanocapsules prepared by the membrane contactor technique.

Sample	Organic phase pressure (bar)	Mean size (nm) ± SD[a]	PdI ± SD[a]	Zeta potential (mV) ± SD[a]	Aggregation	Preparation duration (min)
NC_{19}	0.2	240 ± 39	0.26 ± 0.12	−13.9 ± 3.92	–	6
NC_{20}	0.5	172 ± 14	0.28 ± 0.02	−19.3 ± 9.45	–	4.5
NC_{21}	1	393 ± 9	0.38 ± 0.01	−19.4 ± 2.74	+	1.5
NC_{22}	1.5	–	–	–	++	1.16

[a] $n = 3$; SD: standard deviation between the three assays.

Table 8
The effect of aqueous phase flow rate on the mean size, polydispersity index and zeta potential of vitamin E-loaded nanocapsules prepared by the membrane contactor technique.

Sample	Aqueous phase flow rate (ml min^{-1})	Mean size (nm) ± SD[a]	PdI ± SD[a]	Zeta potential (mV) ± SD[a]	Aggregation	Preparation duration (min)
NC_{23}	260	170 ± 67	0.24 ± 0.06	−12.4 ± 1.34	–	4.15
NC_{20}	700	172 ± 14	0.28 ± 0.02	−17.5 ± 1.10	–	4.5
NC_{24}	1000	212 ± 39	0.38 ± 0.03	−16.2 ± 1.2	–	4.1

[a] $n = 3$; SD: standard deviation between the three assays.

Table 9
The effect of membrane pore size on the mean size, polydispersity index and zeta potential of vitamin E-loaded nanocapsules prepared by the membrane contactor technique.

Sample	Mean pore size (μm)	Mean size (nm) ± SD[a]	PdI ± SD	Zeta potential (mV)	Aggregation	Preparation duration (min)
NC_{25}	0.2[b]	206 ± 48	0.38 ± 10	−17.5 ± 3.8	–	9.9
NC_{20}	0.9[c]	172 ± 14	0.28 ± 0.02	−19.3 ± 8.5	–	4.5
NC_{26}	10.2[c]	205 ± 5	0.4 ± 0.12	−15.2 ± 7.8	–	0.22

[a] $n = 3$; SD: standard deviation between the three assays.
[b] In this case the organic phase was unable to pass with pressure lower than 1 bar.
[c] In this case the used organic phase pressure was 0.5 bar.

membrane pore size on the mean nanocapsule size. The preparation time decreased when using a higher membrane pore size. This result is classical of membranes and porous media transport, as the flow rate through membranes and porous media increases with the mean pore size (Darcy's law).

3.3. Microscopic observation

The vitamin E-loaded nanocapsules were spherical in shape (Fig. 2). No free crystals were detectable. The negative coloration allowed to clearly observe the polymer membrane as a line surrounding the circular droplets in both images.

The microscopic observations show that NCs were spherical (Fig. 3). Some agglomerations can be noticed. This agglomeration was not detectable in the images taken by TEM. Perhaps, they were formed during the preparation of the sample before the microscopic examination. The nanocapsule mean size as observed by TEM correlated well with the mean size obtained using the light-scattering particle size analyzer Malvern Zetasizer (Table 10).

Table 10
Comparison of mean size of nanocapsules determined by Zetasizer and SEM.

Sample	NC prepared at lab-scale	NC prepared at pilot-scale
NC mean diameter measured by Zetasizer (nm)	165 ± 16	172 ± 14
NC mean diameter measured by SEM (nm)	125 ± 17	157 ± 42

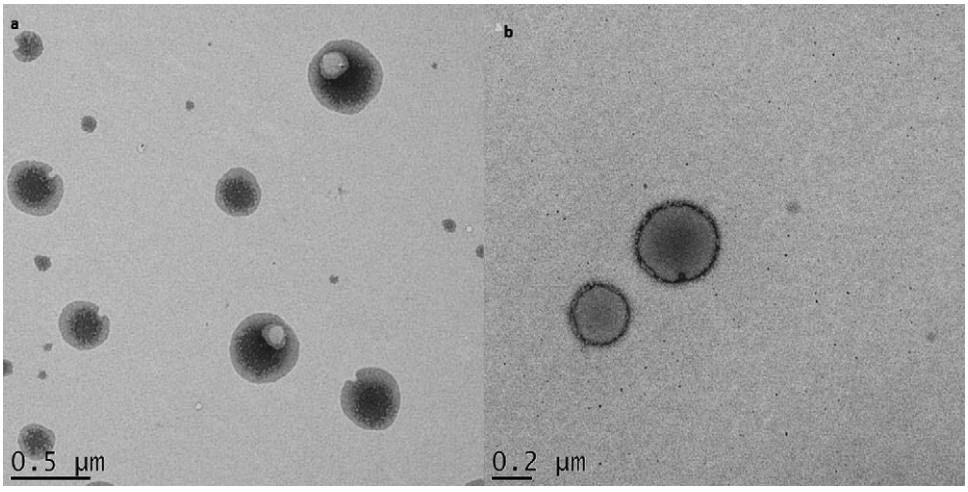

Fig. 2. Transmission electron microscopy of vitamin E-loaded nanocapsules obtained on: a: pilot-scale, b: laboratory-scale.

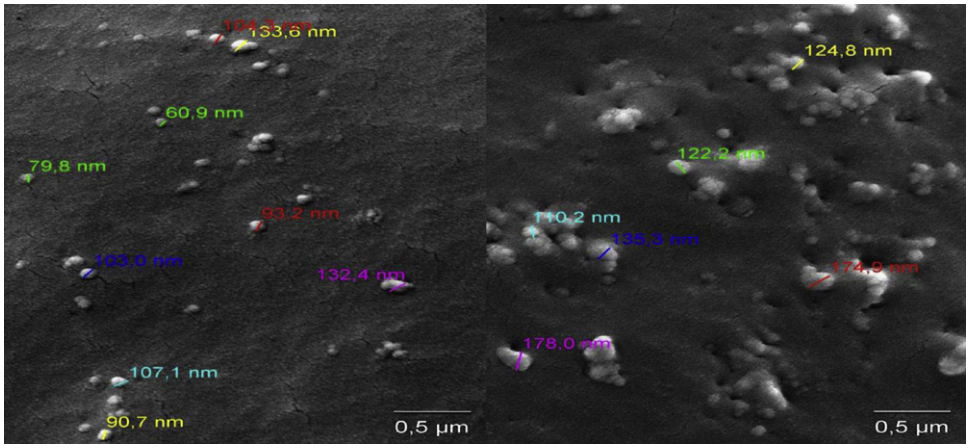

Fig. 3. SEM of vitamin E-loaded NC obtained on: a: lab-scale, b: pilot-scale.

3.4. Encapsulation efficiency

This study was carried out with the optimized formulation obtained at laboratory-scale and with the same scaled-up formulation.

The nanocapsule encapsulation efficiency was the same for the formulation at laboratory-scale (98% ± 1.68) and at pilot-scale (97.65% ± 2.06).

The high encapsulation efficiency of vitamin E in nanocapsules is believed to be due to its oily properties. Drugs with good solubility in oils also show high encapsulation efficiencies. Fresta et al. (1996) reported that the percentage of encapsulation is generally related to the solubility of the drug in the oily inner core.

The high encapsulation yield of α-tocopherol could be considered as an indirect measurement of the membrane permeability and an estimate of the ability of the polymer to protect the internal content of the nanocapsules (Bouchemal et al., 2005).

4. Conclusion

The present study investigated the preparation of vitamin E-loaded nanocapsules by the nanoprecipitation method, at laboratory-scale and pilot-scale. At laboratory-scale, different parameters were tested in order to obtain an optimized formulation. Nanocapsules were obtained with a small mean size (165 nm) and high encapsulation efficiency (98% ± 1.68). The scale-up at the pilot-scale was performed using a membrane contactor. The technique allowed the preparation of vitamin E-loaded nanocapsules with a slight increase in size (172 nm) and with no reduction in drug encapsulation efficiency (97% ± 2.06).

Finally, it can be concluded that the pilot-scale production of vitamin E-loaded nanocapsules prepared by the nanoprecipitation method was possible in an easy and reproducible way. The optimized parameters at pilot scale (organic phase pressure: 0.5 bar, aqueous phase: 700 ml min^{-1}, mean membrane pore size: 0.9 µm) produced vitamin E-loaded nanocapsule with an average mean size of 172 ± 14 nm. Further study of accelerated stability of loaded nanocapsules has been preformed for six months. The NC were almost stable, no significant changes in mean size, zeta potential values and entrapment efficiency were detected.

A pharmacokinetic study of the preparation (in vivo, in vitro drug release) should be carried out to evaluate the difference between the vitamin E-loaded nanocapsules and the traditional suspension forms.

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