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Development of α-Tocopherol Acetate Nanoparticles: Influence of Preparative Processes

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We studied different methods of preparing α-tocopherol acetate (ATA) nanoparticles, which are to be used in targeting the lungs as aerosols in order to prevent cigarette smoke toxicity. Poly-(lactide) nanoparticles were prepared using nanoprecipitation and solvent evaporation techniques, which produced, respectively, too small and too large nanoparticles to be aerosolized. The emulsification-diffusion method produced 2 months stable nanoparticles with a size between (500–700 nm). Increasing ATA concentration (1–7 mg/mL) induced a decrease in the association rate (97–93%) and in the adsorbed ATA rate (7–4.5%), which was associated with variations of Zeta potentials (–27.5 to –24.3 mV) and decrease in polymeric wall thickness and density.

Keywords cigarette smoke toxicity; vitamin E; α-tocopherol acetate; nanoparticles; emulsification diffusion

INTRODUCTION

Cigarette smoke toxicity is characterized by several pulmonary disorders, which are mainly caused by oxidative phenomena (Hautamaki, Kobayashi, Senior, & Shapiro, 1997; Pacht, Kaseki, Mohammed, Cornwell, & Davis, 1986; Scherrer-Crosbie et al., 1996; Schmekel et al., 1992). Therefore, antioxidant agents such as vitamin C (Panda, Chattopadhyay, Ghosh, Chattopadhyay, & Chatterjee, 1999) or vitamin E (Chow, Chen, Thacker, & Griffith, 1984) could be used to prevent this toxicity.

A vitamin E-deficient diet caused a 90-fold depletion of α-tocopherol in lung tissues and resulted in a significant decline of other antioxidants (GSH, ascorbate) as well as accumulation of lipid peroxidation products (Shvedova et al., 2007) and led to higher mortality levels (Chow et al., 1984) in animal models.

Nevertheless, the oral (Scherrer-Crosbie et al., 1996) or intravenous administration of α-tocopherol acetate (ATA) liposomes failed to restore the bronchoalveolar level of vitamin E, which remained inferior in smokers than in non-smoker subjects (Kato et al., 1993; Losowsky, Kelleher, & Walker, 1972). Conversely, the intratracheal administration of ATA liposomes, in animal models, prevented the pulmonary toxicity of various oxidant agents such as paraquat (Suntres, Hepworth, & Shek, 1992; Suntres & Shek, 1995a) and phorbol-myristate (Suntres & Shek, 1995b).

However, liposomes in general have a modest encapsulation capacity <50% (Zaru, Mourtas, Klepetsanis, Fadda, & Antimisiaris, 2007), which requires the development of another system with a higher capacity to encapsulate oils such as vitamin E, the nanocapsules.

The aerosolization remains the only non-invasive way to administer such system; therefore, our hypothesis was that the aerosolization of freeze-dried nanoparticles, with adequate size and high loading capacity for oily compounds, could be an effective drug carrier for pulmonary administration of ATA as suggested by Dailey et al. (2003).

To reach the airways, aerosolized particles with aerodynamic diameters of 1–5 μm are most efficiently deposited in the lower respiratory tract (Bosquillon et al., 2004; Johnson, 1989; Taburet & Schmit, 1994). Nevertheless, engineering an effective formulation of nanoparticles for inhalation therapy (aerosols) must take into consideration many factors such as aggregation during aerosolization because of the hydrophobic surface of nanoparticles, density, aerodynamic diameter, and the release kinetics at the pulmonary level

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(Fiegel, Fu, & Hanes, 2004; Fu, Fiegel, Krauland, & Hanes, 2002).

The aim of our study was to develop a nanoparticles system with specific characteristics such as size, density, and encapsulation capacity, which would permit to target the lungs, and to evaluate the parameters influencing the physicochemical properties of the nanoparticles containing ATA using different preparative techniques.

MATERIALS AND METHODS

Materials

Poly dl-lactic acid (PLA, Resomer® R207, MW: 200,000) was purchased from Boerhinger-Ingelheim (Ingelheim, Germany). Soybean lecithin (Lipoid S75) was a gift from Lipoid Gmbh (Ludwigshafen, Germany).

ATA was purchased from Sigma (Sigma-Aldrich chemie Gmbh, Stenheim, Germany).

The non-ionic stabilizing agents used were poloxamer 188 (Symperonic® PE/F68) (Imperial Chemical Industries, Middlesbrough, England) and polyvinyl alcohol (MW: 30,000–70,000) (Sigma, Germany).

All solvents used were high-performance liquid chromatograph (HPLC) analytical grade.

Methods

Nanoparticles Preparation

Nanoparticles were prepared following three different techniques: nanoprecipitation, solvent-evaporation, and emulsification-diffusion method.

Preparation of Nanoparticles According to the Nanoprecipitation Technique (Fessi, Devissaguet, & Puisieux, 1988). About 125 mg of PLA was dissolved in 25 mL of acetone containing 10–300 mg of ATA. Then, 125 mg of Lipoid S75 was added and dissolved by increasing the temperature to 60°C. The resulting organic solution was slowly poured under magnetic stirring (300 rpm) into 50 mL of 0.5% (wt/vol) poloxamer aqueous solution leading to nanoparticles formation. The acetone and about 40 mL of water were removed under reduced pressure (60°C, 800 hPa) using a rotatory evaporator. The remaining aqueous phase was adjusted to 10 mL in order to obtain suspensions containing final ATA concentrations from 1 to 30 mg/mL.

Preparation of Nanoparticles Following the Solvent Evaporation Process (Venier-Julienne, Vouldoukis, Monjour, & Benoit, 1995). About 100 mg of ATA and 320 mg of PLA were dissolved in 32 mL of dichloromethane. The mixture was emulsified in 100 mL of a 0.5% (wt/vol) polyvinyl alcohol solution using a high-speed homogenizer (Ultra-turrax T25; Janke and Kunkel, IKA® labotechnik, Staufeni, Germany). The emulsification process was carried out for 10 min at 24,000 rpm. Dichloromethane was removed under reduced pressure using a rotatory evaporator (40°C, 700 hPa).

Preparation of Nanoparticles According to the Emulsification-Diffusion Method (Quintanar-Guerrero, Fessi, Alléman, & Doelker, 1996). About 200 mg of PLA was dissolved in 10 mL of ethyl acetate containing 10 mg/mL of ATA. This organic solution was emulsified with 20 mL of a 5% (wt/vol) poloxamer aqueous solution using a high-speed homogenizer for 10 min and 80 mL of water was then added to allow the diffusion of ethyl acetate into the aqueous phase, which led to the formation of nanocapsules. The solvent was removed under reduced pressure (60°C, 800 hPa) and the concentrate was adjusted with 2.5% poloxamer solution to obtain a final volume of 100 mL.

Influence of Preparative Variables on Particles Characteristics Obtained by Emulsification-Diffusion

The Influence of ATA Concentration. Four preparations containing 1, 3, 5, and 7 mg/mL of ATA were prepared as previously described under a stirring rate of 8,000 rpm.

A stability study, for all four preparations, was performed at 25°C for 2 months.

The Influence of Stirring Rate. Several stirring rates (8,000, 13,500, and 24,000 rpm) were tested for the 1 mg/mL ATA preparation.

The Influence of PLA Concentration. About 2, 3, and 5 mg/mL of PLA were evaluated at 8,000 and 24,000 rpm for the 1 mg/mL ATA preparations.

The Influence of Poloxamer Concentration. Suspensions containing 0.5, 1, 2, and 3% wt/vol of poloxamer and 1 mg/mL of ATA were prepared at 8,000 rpm.

The Influence of the Cconcentration Rate. About 1 mg/mL ATA suspensions, prepared at 8,000 rpm, were concentrated under reduced pressure (60°C; 800 hPa) and adjusted with 2.5% poloxamer solution to obtain final volumes of 100, 50, 20, and 10 mL.

The Influence of Diffusion Water Volume. Ethyl acetate was extracted by 0, 30, or 80 mL of water at 8,000 rpm. ATA final concentrations were 5, 2.5, and 1 mg/mL, respectively.

Nanoparticles Characterization

Particle Size Analysis. Particle size was determined by photon correlation spectroscopy using a Coulter N4+ nanosizer (Coultronics, France) at a fixed angle of 90°, at 20°C. The size of microparticles obtained by solvent evaporation was measured by optic microscopy using an ocular micrometer. At least 200 particles were counted to estimate the mean size and the standard deviation.

Zeta Potential Analysis. The electrophoretic mobility and zeta potential measurements were performed with a zetasizer® (Malvern instruments, England).

Determination of ATA Concentrations. ATA concentrations were determined using HPLC analysis. Each sample, dissolved in the mobile phase, was injected onto a reverse phase C_{18} column (Hypersil, 5 μm, 250 × 4.6 mm i.d., Shandon, France) fitted to a guard column (ODS-5μ-C_{18}). The mobile

phase was a mixture of acetonitrile and ethanol (60:40, respectively) at a flow rate of 1 mL/min. Detection was carried out at 284 nm. Data were acquired and processed using the Winilab II chromatography manager (Perichrom, Saulx les chartreux, France).

Determination of the Associated ATA Particles and the Encapsulated ATA Particles. The encapsulated ATA levels (ATA_E) were determined by removing the non-encapsulated (free) (ATA_F) and the loosely surface-bound drug (ATA_B) by exclusion gel chromatography. A 500-µL aliquot of the nanoparticle suspension was passed through a Sepharose CL4B column (1.5 × 20 cm, Pharmacia Biotech), eluted at 1 mL/min with 2.5% poloxamer 188 aqueous solution and the initial turbid fractions containing nanoparticles were pooled. The ATA_E (µg/mL) was determined using HPLC in 100 µL aliquot after dissolving the polymer membrane in acetonitrile and diluting to 10 mL in the mobile phase.

The ATA_F concentration was determined after centrifugation of 1 mL aliquot of the suspension at 100,000 × *g* for 1 h. Then, 100 µL of the supernatant was diluted to 10 mL with a mixture of acetonitrile and water (80:20 vol/vol, respectively) and analyzed using HPLC to determine the non-associated ATA concentration. Total ATA was determined as previously described.

Determination of Nanoparticles Density. Nanoparticles density was determined by isopycnic centrifugation in a 1–60% linear sucrose gradient (50 mM Tris buffer pH 7.4, 2.5 mM EDTA). About 100 µL of the nanoparticle suspension was loaded on the gradient top and the tube was centrifuged at 15,000 × *g* for 1 h at 4°C using a 30 fixed-angle rotor leading to a sedimentation band. The density *D* (g/cm^3) was calculated according to the following equation:

$$D = (d_{60\%} - d_{1\%}) x \frac{D_R}{H} + d_{1\%}$$

where $d_{60\%}$ and $d_{1\%}$ are the densities of 60 and 1% sucrose solution (g/cm^3); D_R is the distance between the top of the gradient and the middle of the nanoparticle's sedimentation band (mm), and *H* is the distance between the top and the bottom of the gradient (mm).

Estimation of the Nanocapsules' Envelop Thickness. Nanocapsules' wall thickness (*WT)* estimation was based on the assumption that nanocapsules are composed only of a polymer wall and an oily core corresponding to the incorporated ATA.

Thus, the calculated density of a nanocapsule D_{cal} is:

$$D_{cal} = (f_{ATA} \times D_{ATA}) + (f_{PLA} \times D_{PLA})$$

where f_{ATA} and f_{PLA} are the volume fractions of ATA and PLA. D_{ATA} and D_{PLA} are the densities of ATA (0.9533 g/cm^3) and PLA (1.250 g/cm^3).

– The respective volume (V) fractions per milliliter of the suspension were calculated according to the equation:

$$f_{ATA} = \frac{V\mathrm{ATA}_E}{V\mathrm{ATA}_E + (M_{PLA} / D_{PLA})} \text{ and } (f_{PLA} = 1 - f_{ATA})$$

where the volume of encapsulated ATA is calculated by dividing the measured ATA concentration by the density and M_{PLA} is the mass of PLA incorporated into the nanoparticles (*Pi-Pf*), as defined above.

– The number of nanoparticles per milliliter of suspension (N) was estimated according to

$$N = \frac{f_{ATA} \times V\ \mathrm{ATA}_E}{V_p}$$

where *V*p is the mean volume of a nanoparticle calculated from the mean radius *R*p (nm) measured by nanosizer analysis.

– The radius of the internal oily core *R*o (nm) is calculated as following:

$$Ro = \sqrt[3]{\left(\frac{3}{4} \times \frac{V\ \mathrm{ATA}_E}{D_{ATA} \times \pi \times N}\right)}$$

– Finally, the envelop thickness *WT* (nm) was obtained as

$$WT = Rp - Ro$$

Determination of the Remaining Ethyl Acetate. Total ethyl acetate residual levels were determined by capillary gas chromatography (1022 GC+, Perkin Elmer, Les Ulis, France) for preparations with final volumes: 100, 50, and 20 mL. About 100 µL of nanoparticle suspensions was diluted to 1.5 mL with a mixture of isopropanol and butyl acetate (1:2 vol/vol). About 0.3 µL aliquot of this solution was injected onto a Supelcowax 10® (30 m × 0.53 mm × 0.50 µm ID column). The temperature was maintained at 40°C for 5 min then increased to 200°C with a rate of 10°C/min. The injector and the detector were heated to 250°C and the helium flow rate was of 2.5 mL/min.

Encapsulated ethyl acetate was determined in suspensions with a final volume of 100 mL by headspace analysis. An aliquot of the supernatant air was injected directly into the previously described capillary gas chromatography.

STATISTICAL ANALYSIS

All preparations and experiments were performed in triplicate. Results were expressed as $M \pm SD$. A one-way analysis of variance or a *U*-test was used to compare the influence of various parameters. *P*-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

After comparing the three techniques used throughout this study, we found that the emulsification-diffusion was the technique that met our criteria concerning the size and the association rate of nanoparticles.

The nanoprecipitation technique led to the formation of two sub-type particles, nanospheres and nanocapsules, depending on the initial concentration of ATA (Table 1). The lowest ATA concentration (1 mg/mL) only led to the formation of nanospheres characterized by a poor AR% (25.9 ± 0.4%). Furthermore, a complete disassociation of ATA from the nanospheres occurred after injection into a sepharose column chromatography. This fact suggests a weak binding energy between ATA and the polymer, probably a hydrophobic bound, because the frictional energy involved during the gel exclusion chromatography was sufficient to break the association. Higher initial ATA concentrations led to nanocapsules with significantly higher AR%, about 90%. The association rate slightly decreased to 88% when the ATA concentration increased to 30 mg/mL, probably because of the disassociation of a small amount of the surface-adsorbed ATA. This observation indicates that the maximal encapsulation capacity has not been reached, which is in agreement with a previous study that showed that the encapsulation of benzyl benzoate, an oily core, was not total (Ammoury, 1990).

Both types of nanoparticles produced by this technique exhibited a mean size of about 150 nm (Table 1). Indeed, this small size would allow the nanoparticles to reach the alveoli, but the Brownian movements would exclude any significant deposition of the drug (Newhouse, 1982) and thus induce a massive exhalation. For this reason, the nanoprecipitation technique was abandoned despite the large encapsulation capacity of the nanocapsules (90%).

The solvent evaporation process described by Venier-Julienne et al. (1995) produced microparticles with a mean size of 12.6 ± 4.1 μm and a high association rate of 97.71 ± 0.08%. Despite using the ultrasonic high-speed stirring, the particles' mean diameter could not be reduced. Such large particles would reach the bronchus and the bronchioles but certainly not the alveoli; they would stop in the upper bronchoalveolar airway (Bosquillon et al., 2004).

In fact, the specific delivery of particles to the alveoli level requires a size between 1.5 and 2 μm (Schreier, Gonzalez-Rothi, & Stecenko, 1993; Shek, Suntres, & Brooks, 1994). Unquestionably, the aerosolization of nanoparticles suspensions would enhance their aggregation within the droplets, which is dependant on the nebulizer technology and on the nanoparticle characteristics (Dailey et al., 2003). Studies have shown that the mass median diameters of aerosols generated upon nebulization were (2–15) folds larger than primary geometric particle diameter (McCallion, Taylor, Thomas, & Taylor, 1996; Bosquillon et al., 2004). Therefore, and in order to obtain aerosolized particles of 1.5–2 μm, nanoparticles' mean size should be between 500 and 700 nm, taking into account that nanoparticles freeze-drying is performed without any size alterations (Auvillain, Cavé, Fessi, & Devissaguet, 1989; Roy et al., 1997).

The emulsification-diffusion technique produced nanoparticles of 500–700 nm corresponding to the optimal size. These particles revealed a high association rate of more than 97% for 1 mg/mL ATA, which slightly decreased to 93% when ATA concentration increased to 7 mg/mL (Table 2). These results are in concordance with those obtained by Bouchemal et al. (2004) with ATA in poly(etherurethane) nanocapsules.

A 2-month stability study revealed that the nanoparticle suspensions were stable concerning particles mean size and AR%, which demonstrates the conservation of the particles' integrity and that neither ATA leakage nor disassociation of the adsorbed ATA took place. No ATA hydrolysis was noticed.

Zeta potential measurements confirmed the hypothesis that a fraction of ATA is adsorbed to the nanoparticles' surface. Table 2 summarizes that Zeta potentials varied from –27 mV, for 1 mg/mL suspensions, to –24.3 mV for 7 mg/mL suspensions, which suggests a partial cover to the polymeric negative charges existing on the surface of the nanoparticles by adsorbing the uncharged ATA (Figure 1). Nevertheless, Zeta potentials remained widely negative, which points to the impossibility of saturating the nanoparticles' surface with ATA because of the steric repulsion induced by its long and free aliphatic chains (16 carbons) (Fukuzawa, Ikebata, & Sohmi, 1993).

TABLE 1
The Characteristics of the Nanoparticles Formed by the Nanoprecipitation Method

α-Tocopherol Acetate (mg/mL)	1	5	10	30
Nanoparticles	Spheres	Capsules	Capsules	Capsules
Size ± *SD* (nm)	169 ± 28	150 ± 48	137 ± 43	155 ± 44
AR% ± *SD* (%)	25.9 ± 0.4*	89.37 ± 1.2***	92.8 ± 1.5**	88.19 ± 0.8

AR%:*$p < .0001$, 1 versus 5; 10 and 30 mg/mL.
**$p < .01$, 10 versus 30 mg/mL.
***$p < .05$, 5 versus 10 mg/mL.

TABLE 2
The Influence of α-Tocopherol Acetate Concentration on Nanoparticles Produced by the Emulsification-Diffusion Method

ATA (mg/mL)	1	3	5	7
Size ± *SD* (nm)	554 ± 77	629 ± 43	637 ± 43	643 ± 47
AR% ± *SD* (%)	97.87 ± 0.16	97.69 ± 0.17***	96.2 ± 0.58**	93.40 ± 0.25*
Zeta potential (mV)	−27.5 ± 0.3††	−26.4 ± 0.1†,†††	−25.7 ± 0.2††	−24.3 ± 0.2
Adsorbed ATA (μg/mL)	68.97 ± 9.21‡,‡‡	187.42 ± 21.3‡‡‡	232.76 ± 27.2	300.79 ± 32.6

AR%: *p < .0001, 7 versus 1 and 3 mg/mL.
**p < .001, 5 versus 1 and 7 mg/mL.
*** p < .05, 3 versus 5 mg/mL.
Zeta potential:
†p < .0001, 3 versus 7 mg/mL.
††p < .001, 1 versus 5 and 7 and 5 versus 7 mg/mL.
†††p < .01, 3 versus 5 mg/mL.
Adsorbed ATA: ‡p < .0001, 1 versus 7 mg/mL.
‡‡p < .001, 1 versus 3, 5, and 7 mg/mL.
‡‡‡p < .01, 3 versus 7 mg/mL.

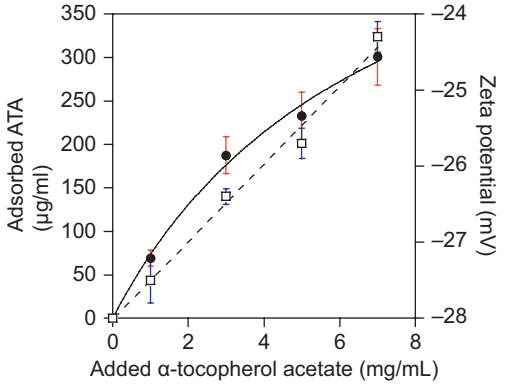

FIGURE 1. The effect of added α-tocopherol acetate (ATA) on zeta potential and the adsorbed ATA. The empty square represents the Zeta Potential (□) and the black circle the absorbed ATA (●).

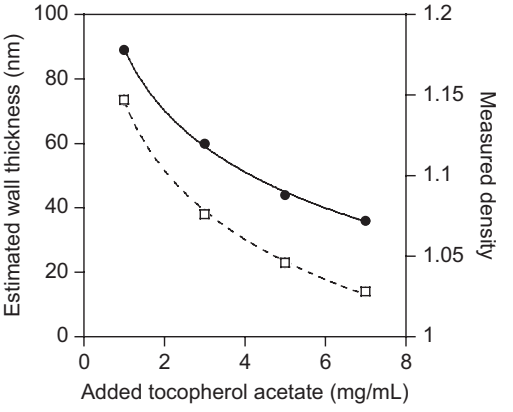

FIGURE 2. The effect of the quantity of α-tocopherol on the measured density and the wall thickness of the formed nanoparticles. The empty square represents the measured density (□) and the black circle the wall thickness (●).

Figure 2 shows that when the quantity of ATA increased both the measured density and the estimated envelop thickness decreased suggesting a modification of the particle's structure; a decrease in the polymeric envelop thickness associated to an increase in the oily core. This decrease of density should not be an obstacle to lyophilization or spray-drying for its values remain high enough to endure such operations (>1 g/cm^3). Furthermore, lyophilizing nanocapsules is not more delicate than liposomes, which is performed successfully nowadays (Zaru et al., 2007).

As expected, increasing the stirring rate from 8,000 to 24,000 rpm was associated with a reduction of the mean size from 554 to 276 nm and a 20% decrease in the encapsulation rate (Table 3). This difference was attributed to the droplet size formed during the emulsification period of the process. Indeed, the oily volume encapsulated in a large particle is higher than this in a small one, which explains the AR% variations. All suspensions were homogenous regardless of the stirring rate employed.

TABLE 3
The Stirring Rate Influence on Emulsification-Diffusion Nanoparticles' Properties

Stirring Rate (rpm)	24,000	13,500	8,000
Size ± *SD* (nm)	276 ± 78	372 ± 46	554 ± 77*
AR% ± *SD*	80.12 ± 5.47	93.08 ± 1.56**	97.87 ± 0.16***

Sizes: *p < .05, 8,000 versus 13,500 and 24,000 rpm.
AR%: **p < .05, 13,500 versus 24,000 rpm.
***p < .01, 8,000 versus 13,500 and 24,000 rpm.

The nanoparticles obtained at 24,000 rpm had a similar size range to the nanoparticles produced at 2,000 rpm in the original method described by Quintanar-Guerrero et al. (1996). In

fact, identical method and materials, save the solvent, were used in our study; therefore, this observation highlights the solvent's effect, which was the only parameter changed in our study. Using propylene carbonate as in the original method (Quintanar-Guerrero et al., 1996) would not have allowed the production of 500–700 nm particles. Therefore, ethyl acetate employment is fully justified because using it at 8,000 rpm led to the formation of nanoparticles with an optimal diameter.

Using 3 or 5 mg/mL of PLA instead of 2 mg/mL, at 8,000 or 24,000 rpm, did not produce any modifications in the nanoparticles' mean diameter. An increase from 80 to 93% in AR% was only observed for 5 mg/mL of PLA and 24,000 rpm (Table 4). When increasing poloxamer concentration from 0.5 to 3% wt/vol, the mean diameter of the nanocapsules decreased from 626 to 302 nm. No AR% variation occurred when using 0.5 or 2% wt/vol poloxamer concentrations, and the AR% was indeed higher than 98%, except for 3% wt/vol poloxamer where preparations presented a significant lower AR% of 90%. Higher poloxamer concentrations were not evaluated because previous works showed that such amounts remained mainly in the external medium and did not play any significant role in the emulsification or the droplet stability (Jalil & Nixon, 1990).

Concentrating suspensions under reduced pressure at 60°C produced large microparticles (Table 5). No AR% variations were observed. Zeta potentials increased while preparing 1 mg/mL ATA preparations then straightly decreased to values close to the ones observed in the unloaded nanoparticles. Variation in zeta potentials is probably related to ATA disassociation occurring at the sustained thermal step. Furthermore, the formation of microparticles seems to indicate an aggregation of the nanocapsules. This aggregation could be explained by the reduction of the final volume, which induced nearness of the nanoparticles (Ammoury, Fessi, Devissaguet, Puisieux, & Benita, 1989; Stolnik et al., 1994), or by an alteration of nanocapsules' surface properties caused by heat. Emergence of aggregates while reducing the final volume justifies keeping it at 100 mL.

Reducing diffusion water volume from 80 to 30 mL led to a large polydispersity without any variations of AR% (Table 6). This was mainly attributed to the alterations of the particles' surface properties rather than the reduction of the final volume.

Ethyl acetate residual rates were also determined for these preparations (Table 7). Reference preparations presented a low residual ethyl acetate rate of 5.61 ± 1.30 ppm, which is 500-folds less than the maximum tolerated limits (Food and Drug Administration HHS, 1997); therefore, these rates were considered acceptable and not toxic. As expected, the ethyl acetate residual rates decreased while reducing the final suspension volume under reduced pressure.

TABLE 4
The Influence of the Increase of PLA Concentration on the Nanoparticles Produced by the Emulsification-Diffusion Method at 24,000 rpm

PLA (mg/mL)	2	3	5
Size ± *SD* (nm)	276 ± 78	279 ± 73	299 ± 40
AR% ± SD	80.12 ± 5.47	79.13 ± 7.40	93.25 ± 2.27*

AR%: *$p < 0.05$, 5 versus 2 and 3 mg/mL.

TABLE 6
Influence of Diffusion Water Volume on the Emulsification-Diffusion Nanoparticles Properties at 8,000 rpm

Diffusion Water Volume (mL)	30	80
Size ± *SD* (nm)	Polydispersed (92%: 535 ± 58 8%: 1,260 ± 230)	554 ± 77
AR% ± SD	97.2 ± 0.31	97.87 ± 0.16

TABLE 5
The Influence of the Concentration Degree on the Nanoparticles Produced by the Emulsification-Diffusion at 8,000 rpm

Final Volume (mL)	100	50	20	10
Size ± *SD* (nm)	554 ± 77	Polydispersed 58%: 596 ± 177, 43%: 2,317 ± 520.5	Polydispersed 74%: 562 ± 131, 26%: 8,686 ± 1,375	Fully polydispersed
AR% ± *SD*	97.87 ± 0.16**	96.89 ± 0.92	98.11 ± 0.13*	97.35 ± 0.25
Zeta p. (mV)	–27.5 ± 0.3††	–30.2 ± 0.4††,††††	–31.3 ± 0.3†††	–33.4 ± 0.3†

AR%: *$p < .01$, 20 versus 10 mL.
**$p < .05$, 100 versus 10 mL.
Zeta potential: †$p < .0001$, 10 versus 20 mL.
††$p < .001$, 100 versus 50 and 20 mL and 50 versus 10 mL.
†††$p < .01$, 20 versus 10 mL.
††††$p < .05$, 50 versus 20 mL.

TABLE 7
The Remaining Ethyl Acetate Rates Measured in the Emulsification-Diffusion Preparations

Final Volume	Total Ethyl Acetate (ppm)
100 mL	5.61 ± 1.30
50 mL	0.273 ± 0.04*
20 mL	<0.16

Total ethyl acetate: *$p < 0.01$, 100 versus 50 mL.

CONCLUSION

This study described the influence of different methods of preparation on the physicochemical properties of ATA nanoparticles. The nanoparticles produced by nanoprecipitation and solvent evaporation methods were, respectively, too small and too large to be retained.

The emulsification-diffusion method produced nanoparticles with optimal size (500–700 nm) and high association rate of ATA. A large evaluation of several preparative variables allowed us to define the optimal formulation parameters.

Our theory of developing a dry aerosol of vitamin E nanocapsules was based on the hypothesis that this molecule could be effective in preventing cigarette smoke toxicity, especially when the bronchoalveolar fluid of smokers was proven to be in deficiency of vitamin E. Sure enough, the fact that vitamin E was effective in preventing the toxicity of other pulmonary oxidants such as paraquat was another factor in supporting our theory.

Still, the freeze-drying followed by the aerosolization of vitamin E nanocapsules remain high techniques and both are difficult to master.

The next step of this study is to verify our hypothesis by intratracheal instillation of suspensions of vitamin E nanocapsules, which were developed through this study, to experimental models of smoking rats in order to investigate the efficiency of this system in preventing the pulmonary toxicity of cigarette smoke. Freeze-drying and aerosolization of the nanoparticles will be performed subsequently and then further tests on animal models will take place in order to judge the final aerosols' efficiency in preventing cigarette smoke toxicity.

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