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Preparation and characterization of spironolactone-loaded nanocapsules for paediatric use

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Abstract

Spironolactone is a steroidal diuretic showing incomplete oral behaviour because of its low solubility and slow dissolution rate. In this study, we applied the nanoprecipitation method to prepare spironolactone-loaded nanocapsules, at laboratory-scale and pilot-scale. The effect of several formulation variables on the spironolactone-loaded nanocapsules properties (average size, drug release rate and drug entrapment) was investigated. The optimized formulations at laboratory-scale and pilot-scale lead to the preparation of spironolactone-loaded nanocapsules with a mean size of 320 and 400 nm, respectively, a high encapsulation efficiency (96.21% and 90.56% respectively), both stable for 6 months. The release of spironolactone from nanocapsules was rapid and complete in a simulated gastric fluid, therefore recourse to spironolactone nanoencapsulation should enhance its oral bioavailability and probably its efficiency. The optimized formulations lead to a high drug-concentration in the liquid preparation (1.5 mg/ml) allowing minimizing the preparation volume administered for children medication.

Keywords: Nanocapsules; Nanoprecipitation; Spironolactone; Formulation; Optimisation; Membrane contactor

1. Introduction

Spironolactone is a specific aldosterone antagonist which is used as a potassium sparing diuretic in premature infants to reduce lung congestion (Atkinson et al., 1988). In neonates oral medication is given through a nasogastric tube making liquid formulations preferable. Furthermore solid dosage forms present problems as children have difficulty swallowing whole tablets or capsules (Standing et al., 2005). Spironolactone is available in both brand (Aldactone®, Pfizer) and generic products in 25, 50 and 100 mg tablet strengths (Hebel, 2006). There is no commercially available oral liquid preparation, but several extemporaneous formulations have been developed (Allen and Erickson, 1996).

As spironolactone is commercially available only in tablet form a number of suspension formulations compounded from tablets (Mathur and Wickman, 1989; Peterson et al., 1989; Nahata et al., 1993), as well as a clear liquid formulation prepared from pure spironolactone (Pramar et al., 1992) have been reported. The problems presented by the poor water-solubility of spironolactone, 28 μg/ml (25 °C) (Sutter and Lau, 1975), have often been solved by using high osmolality syrups as suspending agents (Mathur and Wickman, 1989; Nahata et al., 1993) or high amounts of cosolvents (Pramar et al., 1992), both approaches being undesirable for neonatal patients (Leff and Roberts, 1987). To enhance spironolactone bioavailability, several trials are made in order to improve both its solubility and its dissolution rate. Indeed, it was proven that spironolactone dissolution rate could be enhanced by micronization (Chaumeil, 1998) or by complexation of spironolactone with cyclodextrin (Kaukonen et al., 1997; Soliman et al., 1997).

Nanoparticles represent very promising drug-delivery systems. Nanoparticles regroup both nanocapsules and nanospheres. According to the literature, the nanocapsule corresponds to a polymeric wall enveloping an oil core, whereas the nanosphere consists of a polymeric matrix (Magenheim and

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Benita, 1991; Couvreur et al., 1995). One of the advantages of nanocapsules over nanospheres is their low polymer content and a high loading capacity for lipophilic drugs (Legrand et al., 1999).

Other advantages of confining the drug within a central cavity is that a 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).

The objectives of our work are firstly to optimise the formulation of spironolactone-loaded nanocapsules in terms of drug loading and release, mean size and stability and secondly to scale up this formulation at pilot-scale by increasing 24-fold the laboratory-batch volume from 75 ml to 1.8 l, by using a membrane contactor. This process was reported previously to allow scaling-up and control of nanoparticles size by an appropriate choice of the membrane (Charcosset et al., 2004; Charcosset and Fessi, 2005).

For all spironolactone-loaded nanocapsules preparations, the nanoprecipitation method was used. The nanoprecipitation method is an easy and reproducible method involving dispersion of preformed polymers (Fessi et al., 1989; Govender et al., 1999), based on the interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water from a lipophilic solution. The organic phase (solvent, polymer, eventually oil, and drug) is added dropwise under moderate stirring into the aqueous phase (water, and surfactant).

2. Materials and methods

2.1. Materials

Spironolactone was supplied by Sigma–Aldrich Chemicals.

The oils (Labrafac Hydro®, Labrafil®, Labrafac CC®) were supplied by Gattefosse (France), Myritol® 318 was obtained from CONDEA (France) and the olive oil from Sigma–Aldrich Chemicals.

Polycaprolactone (PCL) with molecular weight of 80 000 and 10 000 were supplied by Sigma–Aldrich Chemicals.

Acetone was obtained from Acros Organics (Belgium), tetrahydrofuran from Laurylab (France) and acetonitrile was supplied by Prolabo (France).

The surfactants Tween® 20, Tween® 80, Span® 20, Span® 80 and Pluronic® F68 were purchased from Fluka Chemika (France).

The oils are of pharmaceutical grade. Polycaprolactone is a polymer used for pharmaceutical preparations and has no reported toxicity. The surfactants are all reported for oral pharmaceutical preparations, and the retained concentrations do not have any reported toxicity for children.

2.2. Preparation of spironolactone-loaded nanocapsules at laboratory-scale

The spironolactone-loaded nanocapsules were prepared using the nanoprecipitation method first developed and described by Fessi et al. (1988, 1989). Spironolactone was dissolved in the oil. PCL and a lipophilic surfactant were dissolved in acetone at 45 °C. The spironolactone in oil was then added to the acetone phase. This organic solution was injected at the rate of 30 ml/min in the aqueous phase containing the hydrophilic surfactant under 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 removed by evaporation at 40 °C under reduced pressure, on a rotavapor, for approximately 30 min. Finally, the nanocapsule suspension was concentrated to a final volume of 10 ml by removal of water under the same conditions.

2.2.1. Spironolactone oil-solubility study

The criteria for selecting the oil are the absence of toxicity, low solubility of the oil in the polymer and vice versa, the absence of risk of degradation of the polymer, and a high capacity to dissolve the drug in question (Couvreur et al., 2002).

A solubility study of spironolactone was carried out in the following oils (Labrafil®, Labrafac® CC, Labrafac® Hydro, Myritol®, and olive oil). Excess amounts of spironolactone were dissolved in 10 ml oil. The samples were sonicated for 30 min at 25 °C, and then shaken under magnetic stirring for 24 h. The suspensions were subsequently filtered through a 0.45 μm membrane filter, followed by dilution with water. The filtered sample solutions were analyzed using an UV–vis spectrophotometer (UV mc^2, Safas, Monaco) at wavelength of 238 nm.

2.2.2. Optimisation of the polymer

PCL with molecular weight of 10 000 and 80 000 were both tested. Spironolactone-loaded nanocapsules were prepared with different amounts of each polymer: 100, 125 and 250 mg, respectively.

The other constituents of formulation were unchanged: 15 mg spironolactone, 0.5 ml Labrafac Hydro®, 75 mg Span® 80, 25 ml acetone, 75 mg Tween® 80 and 50 ml water.

The effect of molecular weight and the amount of PCL was evaluated by measuring the mean size and by observing the absence or presence of aggregates.

2.2.3. Optimisation of the surfactant

The effect of surfactant types and amount was studied with Tween®20/Span® 20, Tween® 80/Span® 80 and Pluronic® F68. The different amounts for Tween®20/Span® 20 and Tween® 80/Span® 80 were respectively 75/75, 150/0 and 75/0 mg and for Pluronic® F68, 50, 150 and 250 mg.

The different formulations were realized with 125 mg PCL 10 000, 25 ml acetone, 15 mg spironolactone, 0.5 ml Labrafac Hydro for the organic phase and 50 ml of water with an hydrophilic surfactant for the aqueous phase.

The effect of surfactant type and amount was evaluated by measuring the mean nanocapsules size.

2.2.4. Optimisation of the aqueous to organic phase volume ratio

The effect of aqueous phase volume was investigated by comparing formulations prepared with 50, 75 and 100 ml water.

The different formulations were carried out with 125 mg PCL 10 000, 25 ml of acetone, 15 mg spironolactone, 0.5 ml Labrafac Hydro in the organic phase and 150 mg Tween® 80 in the aqueous phase.

The effect of organic phase volume ratio was evaluated by measuring the mean nanocapsules size and the encapsulation efficiency of spironolactone.

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

2.3.1. Experimental set-up

The experimental set-up used for the experiments is shown in Fig. 1. It included a pump (PCM, France), a Micro Carbosep/Kerasep crossflow filtration device (Rhodia Orelis, France) equipped with two manometers placed at the module inlet and module outlet, and a valve placed at the module outlet. The aqueous phase was stirred continuously with an impeller RW 20 (Ika-Werk). The organic phase was placed in a pressurized vessel (Millipore), equipped with a manometer, connected to a nitrogen bottle and to the membrane module on the filtrate side. The experiments were conducted at $22 \pm 1\,^{\circ}C$.

The membrane used was a Kerasep ceramic membrane with an active ZrO_2 layer on an Al_2O_3–TiO_2 support (Rhodia Orelis, France). The membrane length was 0.4 m, the inner diameter 6×10^{-3} m and the outer diameter 1×10^{-2} m. Therefore, the active membrane surface was $7.5 \times 10^{-3}\ m^2$. The membrane investigated was a microfiltration membrane with a mean pore size of 0.1 μm.

2.3.2. Nanoparticles preparation using a membrane contactor

The following protocol was used for the experiments, and was the same as described previously (Charcosset and Fessi, 2005). The aqueous phase was stirred continuously and circulates tangentially to the membrane surface. The organic phase was placed in the pressurized vessel. 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 the pressurized vessel to the membrane module. The averaged dispersed phase flux (J) was calculated as the organic phase volume (V) divided by the reaction time (Δt) and the membrane surface (A): $J = V/(\Delta t\, A)$. This preparation was then evaporated under reduced pressure at 40 °C to eliminate the solvent and to concentrate the nanocapsules suspension. At the end of the experiment, a sample in the nanoparticles preparation was taken for size analysis.

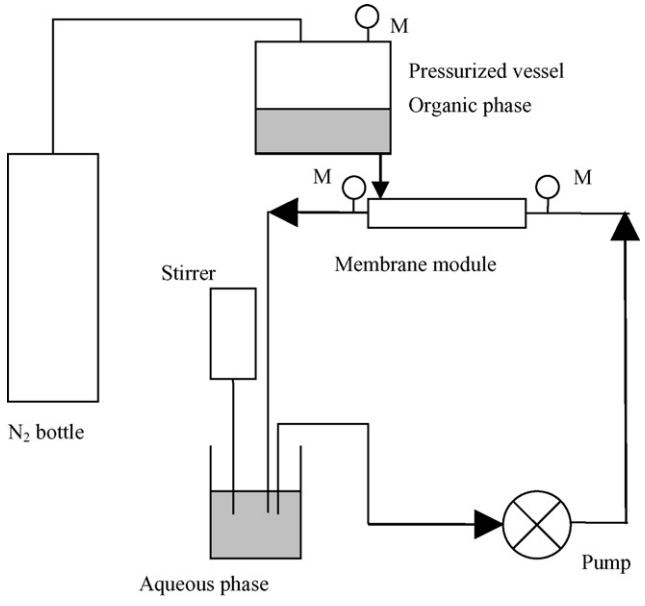


Fig. 1. Experimental set-up for the preparation of nanoparticles at a pilot-scale. M: manometer.

The microfiltration membrane was then regenerated. The washing was performed by flushing the membrane module with 5 l of water circulating in an open cycle, then with 300 ml tetrahydrofuran in the pressurized vessel (pressure 2×10^5 Pa (2 bar)) and 3 l of water circulating tangentially to the membrane surface, and finally with 5 l pure water circulating in a closed cycle for 10 min (transmembrane pressure 2×10^5 Pa (2 bar)). The membrane permeability was measured at the beginning of each experiment and is checked to be around 90% of its initial value. The reproducibility of both laboratory- and pilot-scale preparations were estimated on three experiments, performed at the same operating conditions, to be ±5%.

Three formulations were tested with the membrane contactor (Table 1). For the first one, the optimised formulation obtained on laboratory scale was scaled-up for the membrane contactor (24-fold). The second formulation was calculated with smaller amount of the various constituents (80%) excepting water and acetone. The third formulation was the same as the first one, excepting a higher amount of surfactant (130%).

2.4. Nanocapsules characterization

2.4.1. Size analysis

The mean size of the spironolactone-loaded nanocapsules was measured by a diffusion method using a light-scattering particle size analyser Coulter LS 230 (Beckman Coulter, Coultronics, France).

Table 1
Formulations used for the preparation of spironolactone-loaded nanocapsules at pilot-scale with the membrane contactor

	Constituents	Formulation 1	Formulation 2	Formulation 3
Organic phase	Acetone	0.61	0.61	0.61
	PCL 10000	3 g	2.4 g	3 g
	Spironolactone	360 mg	288 mg	360 mg
	Labrafac Hydro	12 ml	9.6 ml	12 ml
Aqueous phase	Water	1.21	1.21	1.21
	Tween® 80	3.6 g	2.88 g	4.8 g

A sample placed in the fluid module is circulated through a sample cell at constant speed. A beam of laser light shone through the cell is diffracted by particles within the sample, and the forward scattered (or diffracted) light is collected by a series of detectors.

Information about particles smaller than 0.4 μm is limited in diffraction pattern, so another technique is used. Thus, the LS 230 includes another measurement assembly, called polarization intensity differential scattering (PIDS). The PIDS assembly consists of an incandescent light source and polarizing filters, a PIDS sample cell and an additional seven photodiode detectors (six to measure scattered light plus one to monitor the beam strength).

To measure the particle size distribution, 0.5 ml suspension is introduced in the measurement compartment containing 125 ml of water. The results are presented as volume fraction distributions.

2.4.2. Microscopic observation

Transmission electron microscopy (TEM) pictures were taken with CM 120 microscope (Philips, France) operating at 80 kV acceleration. The sample preparation was performed according to similar previous studies (Guinebretière et al., 2002). The spironolactone-loaded nanocapsules were diluted by a factor of 30 and deposited on a copper grid. Negative staining with a 1% sodium phosphotungstate solution was made directly on the deposit.

2.4.3. Encapsulation efficiency

Total spironolactone concentration (T_{SP}) was determined after dissolution of 200 μl of spironolactone-loaded nanocapsules suspension in 10 ml acetonitrile.

Free spironolactone concentration (F_{SP}) was determined after separation of loaded-nanocapsules from the aqueous medium by ultracentrifugation (Optima^{TM} Ultracentrifuge, Bekman-Coulter Instruments, USA). Five millilitres of samples to be measured were centrifuged at 50 000 rpm for 30 min at 20 °C. The free spironolactone concentration was then determined in the supernatant.

The various concentrations were measured at the absorbance of 238 nm with a spectrophotometer UV–vis (UV mc^2, Safas, Monaco). The spectrophotometric method was validated as usually required.

The spironolactone encapsulation efficiency was calculated as follows:

$$\text{encapsulation efficiency}\,(\%) = \frac{T_{SP} - F_{SP}}{T_{SP}} \times 100$$

The encapsulation efficiency was determined in triplicate.

2.4.4. Storage stability

The spironolactone-loaded nanocapsules suspensions prepared on laboratory-scale and pilot-scale were stored at 25 °C during a period of 6 months.

Stability was evaluated by comparing the initial particle size and encapsulation efficiency with those obtained after 6 months storage at 25 °C.

2.4.5. Dissolution studies

The drug release rate from nanocapsules suspension was determined using the 5th edition European Pharmacopoeia type dissolution apparatus (Erweka DT 12R, France). Simulated gastric fluid (SGF, 2 g NaCl, 80 ml HCl 1N and 1000 ml water) was used as the dissolution medium and maintained at 37 ± 0.5 °C at a rotation speed of 60 rpm. The suspension was then diluted in the dissolution medium. Perfect sink conditions prevailed during the drug release studies. Ten millilitres sample was withdrawn at regular intervals, passed through a 0.1 μm membrane filter (Millipore, France), and analysed spectrophotometrically at 238 nm to determine the concentration of drug present in the dissolution medium. The initial volume of the dissolution fluid was maintained by adding 10 ml of fresh dissolution fluid after each withdrawal. All experiments were conducted in triplicate.

3. Results and discussion

3.1. Preparation of spironolactone-loaded nanocapsules at laboratory-scale

3.1.1. Spironolactone oil-solubility study

For the development of spironolactone nanocapsules suspension for oral delivery, a suitable oil need to be chosen.

The maximum spironolactone solubility was determined for each oil retained (Table 2).

The solubility obtained is the lowest for the Myritol® oil (2 mg/ml) and the largest for the Labrafac® Hydro oil (30 mg/ml). Therefore, the Labrafac® Hydro oil (a mixture of C_8/C_{10} ethoxylated glycerides) was retained for the following spironolactone-loaded nanocapsules preparation.

3.1.2. Polymer optimisation

The effect of PCL formulation type and amount on nanocapsule size was represented in Table 3. The smallest particle size of spironolactone-loaded nanocapsules (610 nm) and the absence of aggregates were obtained with 125 mg of PCL 10 000 used. Hence, 125 mg of PCL 10 000 was retained for further studies. We observed that the larger sized nanoparticles were obtained with the higher molecular weight polymer, due to higher organic solution viscosity.

3.1.3. Surfactant optimisation

With the surfactants Tween®20/Span®20 the smallest particle size of spironolactone-loaded nanocapsules was 825 nm (Table 4). In the other hand, with the couple Tween®80/Span®80

Table 2
Solubility of spironolactone in different oils

Type of oil	Maximum solubility (mg/ml)
Labrafil®	5 ± 0.01
Labrafac® CC	7 ± 0.01
Labrafac® Hydro	30 ± 0.02
Myritol®	2 ± 0.01
Olive oil	10 ± 0.02

Table 3
Effect of PCL type and amount on the mean size of spironolactone-loaded nanocapsules and presence of aggregates

Samples	MW of PCL	Amount of PCL (mg)	Mean size (nm) ± S.D.[a]	Aggregates
NC_1	10000	100	741 ± 15	–
NC_2	10000	125	610 ± 11	–
NC_3	10000	250	924 ± 7	++
NC_4	80000	100	952 ± 9	–
NC_5	80000	125	819 ± 21	–
NC_6	80000	250	1145 ± 13	++

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

Table 4
Effect of surfactant on the mean size of spironolactone-loaded nanocapsules

Samples	Surfactants	Amounts (mg:mg)	Mean size (nm) ± S.D.[a]
NC_1		75:75	1300 ± 10
NC_2	Tween20:Span20	150:0	825 ± 8
NC_3		75:0	1100 ± 25
NC_4		75:75	610 ± 11
NC_5	Tween80:Span80	150:0	320 ± 12
NC_6		75:0	552 ± 15
NC_7		50	814 ± 11
NC_8	Pluronic® F68	150	638 ± 7
NC_9		250	725 ± 18

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

the smallest particle size obtained was 320 nm. We observed that the smallest sizes were obtained in the presence of only hydrophilic surfactant, at the proportion of 0.30% (w/v) of the aqueous phase. The addition of a lipophilic surfactant in the organic phase involved an increase in nanocapsules mean size. With the Pluronic® F68, the smallest particle size was obtained with the same proportion of hydrophilic surfactant than the former experiences (0.30%, w/v). Also, the increase or decrease of this amount of surfactant resulted in an increase in nanocapsules mean size. Among all surfactants tested, the Tween® 80 gave the smallest particle size. Hence, 0.30% (w/v) of Tween® 80 was retained for further experiments.

3.1.4. Aqueous to organic phase volume ratio optimisation

The effect of increased aqueous phase volume was determined by comparing the spironolactone-loaded nanocapsules prepared using 50 ml water, 75 and 100 ml water. The results are presented in Table 5.

Table 5
Effect of aqueous phase volume on the mean size and encapsulation efficiency of aqueous to organic phase volume ratio

Volume of water (ml)	Mean size (nm) ± S.D.[a]	Encapsulation efficiency (%), % ± S.D.[a]
50	320 ± 12	96.21 ± 1.03
75	323 ± 8	85.42 ± 1.77
100	536 ± 14	75.93 ± 1.49

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

The mean size of spironolactone-loaded nanocapsules is unchanged with 50 and 75 ml of water (320 ± 12 and 323 ± 9 nm, respectively), but increased with a higher amount of water (536 ± 14 nm with 100 ml water).

The increase in the aqueous phase volume was also associated with lower encapsulation efficiency: 85.42% obtained with 75 ml water and 75.93% with 100 ml water, compared to 96.21% drug recovered with 50 ml water. Our results are in agreement with those of Chorny et al. (2002) who observed a decrease in drug recovered with an increase in the aqueous phase volume for nanospheres prepared by nanoprecipitation.

3.2. Preparation of spironolactone-loaded nanocapsules at pilot-scale with the membrane contactor

The average spironolactone-loaded nanocapsules size obtained at laboratory-scale, for the optimized formulation, was equal to 320 ± 12 nm. The scale-up of this formulation with the membrane technique (formulation 1 in Table 1) gave an average size of 610 ± 13 nm. The second essay realised with the same formulation (80% of the various constituents) and unchanged amounts of solvents, gave a mean size of 560 ± 15 nm. The third formulation with a higher surfactant concentration (+30% from formulation 1) allowed obtaining spironolactone-loaded nanocapsules with a mean size of 400 ± 9 nm. This result was confirmed by Christov et al. (Christov et al., 2002) who observed a decrease of the drop size of emulsions, prepared by the membrane technique, when the concentration of Tween 20 was increased. They explained this fact with a greater dynamic contact angle solid–water–oil.

The organic phase flow-rate measured during the manipulations is equal to 3.2 $m^3/h\,m^2$. This flow-rate corresponds to the preparation of 1.8×10^{-3} m^3 of nanocapsules suspension in 1.5 min, which confirms the potentially of this process for industrial applications.

3.3. Microscopic observation

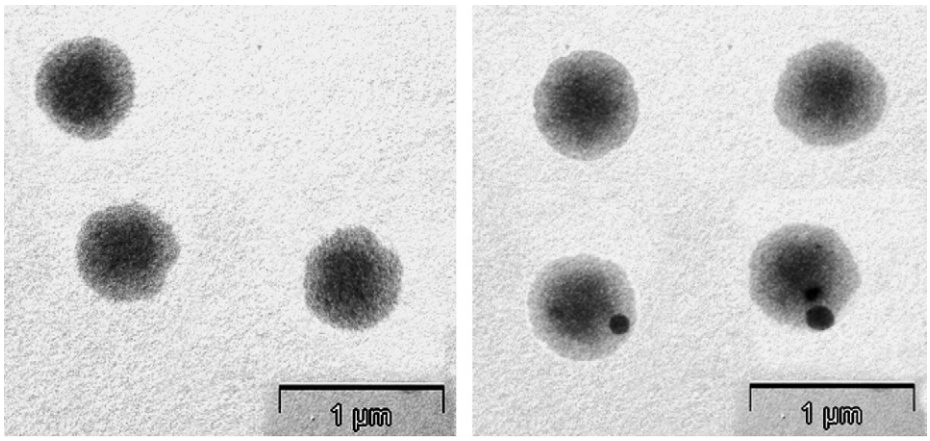
The spironolactone-loaded nanocapsules were spherical in shape (Fig. 2). No free crystals were detectable. The nanocapsules size as observed by transmission electron microscopy correlated well with the size measured by the diffusion method using a light-scattering particle size analyser Coulter LS 230.

3.4. Encapsulation efficiency

This study was carried out with the optimized formulation obtained at laboratory-scale and with formulation 2 (Table 1) at pilot-scale.

The percentage of nanocapsules encapsulation efficiency was slightly higher with the formulation at laboratory-scale (96.21% ± 1.03) than at pilot-scale (90.56% ± 1.24). This result is in agreement with that reported by Galindo-Rodriguez et al. (2005) who observed a slight reduction in the Ibuprofen loading capacity of nanoparticles from laboratory-scale to pilot-scale.

The high encapsulation efficiency of spironolactone in nanocapsules is believed to be due to its good solubility in



(a) (b)

Fig. 2. Transmission electron microscopy of: (a) spironolactone-loaded NC obtained on laboratory-scale and (b) spironolactone-loaded NC obtained on a pilot-scale.

Labrafac Hydro like demonstrated by Fresta et al. (1996) who reported that the percentage of encapsulation is generally related to the solubility of the drug in the oily inner core.

3.5. Storage stability

After storage for 6 months at 25 °C, the spironolactone-loaded nanocapsules displayed only a slight increase of particle size from 320 to 332 nm for the nanocapsules prepared at laboratory-scale and from 400 to 410 nm for the nanocapsules prepared at pilot-scale. For both nanocapsule preparations, the initial encapsulation efficiency was maintained and no nanoparticule aggregation was observed during storage.

This result shows that both nanocapsule dispersions possess a good storage stability, due an adequate proportion of different constituents in the preparations.

3.6. Dissolution study

In sink conditions, the release of spironolactone from nanocapsules was very rapid and complete, as 100% of the drug was released after 20 min from nanocapsules prepared at laboratory-scale and after 15 min from nanocapsules prepared at pilot-scale (Table 6). This slight difference of release speed may be due to the higher surfactant amount used for the pilot-scale preparation. Indeed, Xiaohong et al. (1999) have demonstrated that a significant increase in the release rate of *Leptospira interrogans* antigens from poly-D,L-lactide-poly (ethylene glycol) microspheres was obtained when a higher concentration of Tween® 80 was introduced in the aqueous phase.

Table 6
Percentage of dissolved spironolactone from laboratory-scale and pilot-scale preparations

Time (min)	Percentage of dissolved spironolactone (%) ± S.D.[a]	
	Laboratory-scale	Pilot-scale
5	65.13 ± 1.52	86.27 ± 0.53
10	80.62 ± 0.74	98.6 ± 0.78
15	93.4 ± 2.64	100.35 ± 1.15
20	100.51 ± 1.78	100.71 ± 0.95
30	101.5 ± 0.58	101.02 ± 1.53

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

3.7. Optimized formulations at laboratory-scale and pilot-scale

The optimized formulations and their main characteristics are summarized in Table 7.

Table 7
Comparison of the results obtained with the laboratory and pilot scales

Organic phase	Aqueous phase	Mean size (nm) ± S.D.[a]	Encapsulation efficiency (%) % ± S.D.[a]	Storage stability
Formulation at laboratory-scale Acetone: 25 ml PCL 10000: 125 mg Spironolactone: 15 mg Labrafac Hydro: 0.5 ml	Water: 50 ml Tween 80: 150 mg	320 ± 12	96.21 ± 1.03	6 months stable
Formulation at pilot-scale Acetone: 0.6 l PCL 10000: 3 g Spironolactone: 360 mg Labrafac Hydro: 12 ml	Water: 1.2 l Tween 80: 4.8 g	400 ± 9	90.56 ± 1.24	6 months stable

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

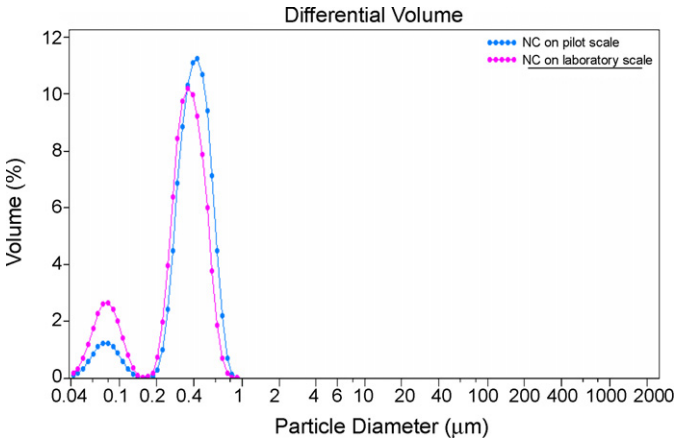


Fig. 3. Size distribution of spironolactone-loaded nanocapsules prepared on laboratory and pilot-scales.

The addition of 30% of surfactant allowed obtaining spironolactone-loaded nanocapsules with the smallest particle size, when we scaled up the laboratory formulation to the pilot-scale. The two preparations are stable for 6 months and a slight decrease of the encapsulation efficiency was observed with the pilot-scale.

Fig. 3 shows the particle size distribution of spironolactone-loaded nanocapsules prepared on laboratory-scale and pilot-scale (membrane contactor). The two distributions are very similar.

4. Conclusion

The present study investigated the preparation of spironolactone-loaded nanocapsules by the nanoprecipitation method, at laboratory-scale and pilot-scale. At laboratory-scale, different parameters were tested in order to obtain an optimised formulation. Nanocapsules were obtained with small mean size, high encapsulation efficiency, rapid and complete release and successfully stability for 6 months. Moreover the concentration of spironolactone in the liquid preparation (1.5 mg/ml) was appropriate for oral children administration. The scale-up at the pilot-scale was performed and allowed production of spironolactone-loaded nanocapsules with a slight increase in size and a slight reduction in drug encapsulation efficiency. Finally, it can be concluded that pilot-scale production of spironolactone-loaded nanocapsules prepared by the nanoprecipitation method was possible and allowed production of nanocapsules in an easy and reproducible way. This study is intended to be completed by an organoleptic evaluation of the spironolactone-loaded nanocapsules, together with a sweetening and flavouring study. A pharmacokinetic study of the preparation should also be carried out to evaluate the difference between the spironolactone-loaded nanocapsule and the suspension forms.

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