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RESEARCH PAPER

Project, Design, and Use of a Pilot Plant for Nanocapsule Production

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ABSTRACT

The aim of this study was to find the scale-up parameters necessary for the preparation of nanocapsules (NCs) for pharmaceutical purposes. Starting from the laboratory scale (0.06 L), we designed and assembled a pilot plant (2 L) to produce NCs with the so-called emulsification-diffusion technique. We wanted to check if classical tools adequate for the pharmaceutical industry and for industrial scale-up purposes according to well-known chemical engineering technique could be used to perform the NC preparation. Experiments were carried out by varying some operative parameters, such as the impeller speed, the agitation duration for the emulsion preparation, and the reagent concentrations. As expected, good accordance between the NC produced at the laboratory scale and at the pilot plant scale was obtained. We conclude that the pilot plant can be used to perform a scale-up study of the industrial production of NC.

Key Words: *Encapsulation; Nanocapsules; Pilot plant; Scale-up*

INTRODUCTION

Numerous studies in the last 20 years have shown that a good way to enhance drug action is to associate the active molecule with a carrier system. Nanoencapsulation techniques result in products containing coated particles that act as drug delivery systems. The encapsulation allows enhancement of the drug stability by protecting it from its environment (storage conditions) and reducing adverse or

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toxic effects. Another objective of delivery systems is to control the site of action, as well as the kinetics of release of drug active molecule.

The first systems developed were first-generation microparticles, but research in the past decade addressed the development of nanoparticulate (NP) delivery systems that are also intended for intravenous injection use.

The interest in these colloidal vectors is to enhance the therapeutic effect by targeting the active molecule to its site of action and by creating a high local concentration. The pharmacokinetic profile of the drug is then modified; the efficiency is increased, while the amount of drug administrated and the risks of side effects are decreased (1). Thus, the NP can be considered as a successful alternative to other forms for carrying active molecules. In fact, the NP possess better stability in biological fluids (2,3) during storage, and their preparation is more suitable to scaling up.

The emulsification-diffusion technique, patented by Quintanar-Guerrero and Fessi in 1999 (4), is used to produce nanocapsules (NCs) based on biodegradable polymers. This new method involves the use of a partially water soluble solvent, which is previously saturated with water to ensure the initial thermodynamic equilibrium between the two liquids (water and solvent). Polymer, oil, and drug are dissolved in the saturated solvent, producing the organic phase. This organic phase is then emulsified, under vigorous agitation, in an aqueous solution containing a stabilizer agent. The obtained emulsion presents size values between 0.4 μm and 1.3 μm and is stable at room temperature for at least 5 months (no coalescence or breakdown detected). The subsequent addition of water to the system, under moderate stirring, causes solvent diffusion into the external phase, which induces the interfacial deposition of the polymer to form the NCs (5). Depending on its boiling point, the solvent can be eliminated by distillation or ultrafiltration.

This technique, already under study at the laboratory scale, has been tested using a pilot plant designed for this purpose. The requirement was to construct a pilot plant to produce NC efficiently and to obtain the scale-up parameters for an industrial production.

As the pilot plant was assembled, it was tested to prepare NCs. The mechanism of NC formation was evaluated under different preparation conditions to control the process. Some of the parameters involved were the impeller rotational speed, the agitation duration necessary for the emulsion preparation, and the reagent type and concentration. The parameters involved for the emulsion dilution in distilled water were the impeller rotational speed and the agitation time. Last, we tried to reach the maximum evaporation rate for the evaporation step keeping the stability of the system and the size of the NC produced.

EXPERIMENTAL

Material

Two polymers were used for the preparation of the NCs: PCL (poly-ε-caprolactone), purchased from Aldrich Chemical Company, Inc., and Eudragit® E100, a copolymer of acrylic acid and methacrylate purchased from Röhm GmbH-Chemische Fabrick.

The core of the NC was made of an oil, Miglyol® 812, a mixture of capric and caprylic triglycerides from Condea Chemie GmbH. For the tests of drug encapsulation, indomethacin (1-[*p*-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid) from Sigma was chosen as the active principle in solution in the oil.

Pure ethyl acetate (Laurylab) was used as a partially water miscible solvent because of its widely recognized low toxicity, good solubilizing properties, and low boiling point.

The continuous phase of the emulsion was constituted of distilled water saturated with ethyl acetate. The distilled water was purified with a Millipore alpha-Q system (Millipore SA). PVAL (polyvinyl alcohol, Mowiol® 4-86) was selected as a stabilizer agent for the emulsion preparation and the dispersion stabilization because of its good solubility, its suitability for ingestion, and its compatibility with the system. It was supplied by Hoechst.

Through a mass balance calculation, the flow sheet presented in Fig. 1 was estimated (6). The same percentages by weight for reagent concentrations were used in both laboratory and pilot plant scales.

Steps

The different steps of the emulsion/diffusion technique are presented in the flow sheet (Fig. 1) and

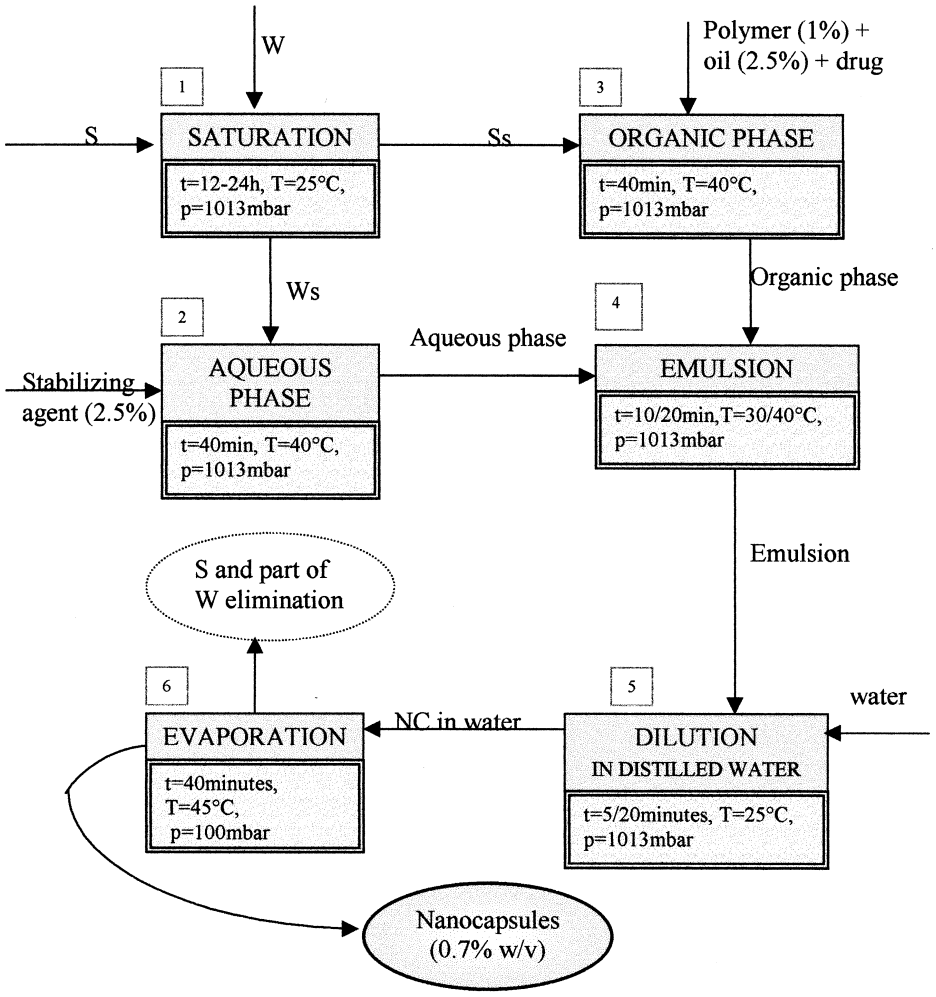

Figure 1. Flow sheet: P, polymer; S, pure solvent; Ss, solvent saturated with water; W, pure water; Ws, water saturated with solvent.

can be described as follows:

1. Solvent and water mutual saturation.
2. Preparation of the aqueous phase: The stabilizing agent is dissolved in water saturated with the solvent.
3. Preparation of the organic phase: The polymer, oil, and drug mixture is dissolved in the solvent saturated with water.
4. Emulsification (oil in water) using a high-speed drive unit.
5. Addition of the emulsion to the distilled water, under moderate stirring, with subsequent formation of NCs.
6. Elimination of the solvent and part of the water by evaporation under reduced pressure or by crossflow filtration (ultrafiltration).

Equipment

Table 1 shows the equipment scale-up from the laboratory scale (volume of 0.06 L) to the pilot scale (volume of 2 L) that allowed us to carry out the experimental steps described above.

The plant layout presented in Fig. 2 shows the pilot plant used to perform the emulsion-diffusion method following the procedure described below.

Table 1

Equipment for the Emulsification/Diffusion Technique

	Equipment			
	Laboratory		Pilot	
Step	Reactor	Agitation	Reactor	Agitation
1. Saturation	Settling flask	Manual	$V = 2.5$ L	Turbine, 4 paddles
2. Preparation of the aqueous phase	Round-bottom flask, $V = 0.1$ L	Magnetic	Jacketed for heat fluid circulation, $V = 2.5$ L	Turbine, 4 paddles
3. Preparation of the organic phase	Beaker, $V = 0.3$ L	Turbine	Jacketed for heat fluid circulation, $V = 2$ L	Turbine, 4 paddles
4. Emulsification	Beaker, $V = 0.15$ L	Ultra Turrax T25	Jacketed for heat fluid circulation, $V = 2$ L	Ultra Turrax T50 + turbine, 4 paddles
5. Dilution	Beaker, $V = 0.3$ L	Turbine	Reactor, $V = 6$ L	Turbine, 6 paddles
6. Evaporation	Rotovapor		Rotovapor	

Procedure

In Fig. 2, the reactor R1 was used for the preparation of the saturated phases. The reagents were mixed, and the resulting mixture was allowed to rest to achieve the mass transfer process and reach the thermodynamic equilibrium between the phases. The lower phase (the aqueous one) was then poured into the reactor R2 using the valve V1. In the reactor R1, the organic phase was prepared by adding the polymer, the oil, and the drug (only for encapsulation tests) and maintaining them under agitation and heating at 40°C for about 40 min.

The preparation of the aqueous phase was performed in the reactor R2 by dissolution of the PVAL under agitation and heating at 40°C. The emulsion was prepared in the same reactor R2 under vigorous agitation using the rotor-stator mixer as a high-speed dispersing and emulsifying unit. No heating was required during the emulsification.

The reactor R3 was used for the dilution of the emulsion under moderate agitation with subsequent formation of the NC. The NC suspension was then transferred into the storage tank R4 using the valve V2.

The pilot plant was designed without pumps to avoid emulsion breakdown. The circulation of the products from one reactor to another was induced only by gravity. Therefore, the reactors were connected in series using PTFE (Teflon) pipes.

PHYSICAL CHARACTERIZATION

The mean size and polydispersity were measured using a Coulter LS series instrument (Beckman Coulter Company, Coultronics France S.A.) with a fluid model; the instrument can measure sample particles suspended in a liquid. This instrument measures the size distribution of particles (from 0.04 μm to 2000 μm) using the laser light diffraction by particles as the main source of information about particle size. Information about particles smaller than 0.4 μm is limited in diffraction patterns, so the LS230 includes another measurement assembly called the PIDS (polarization intensity differential scattering). 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). The measurements were made according to the following procedure: The dispersed particles are placed into the vessel containing the suspension fluid. The mixture flows through the sample cell and hoses in a closed-loop system. Emulsions prepared by the emulsion-diffusion technique were analyzed using water saturated with solvent as the suspension fluid to avoid the diffusion of the solvent (and therefore the formation of NCs) during the analysis.

Otherwise, the NC size analysis was carried out by dilution of the NC suspension in distilled water. When it was possible, the measurement was repeated three times.

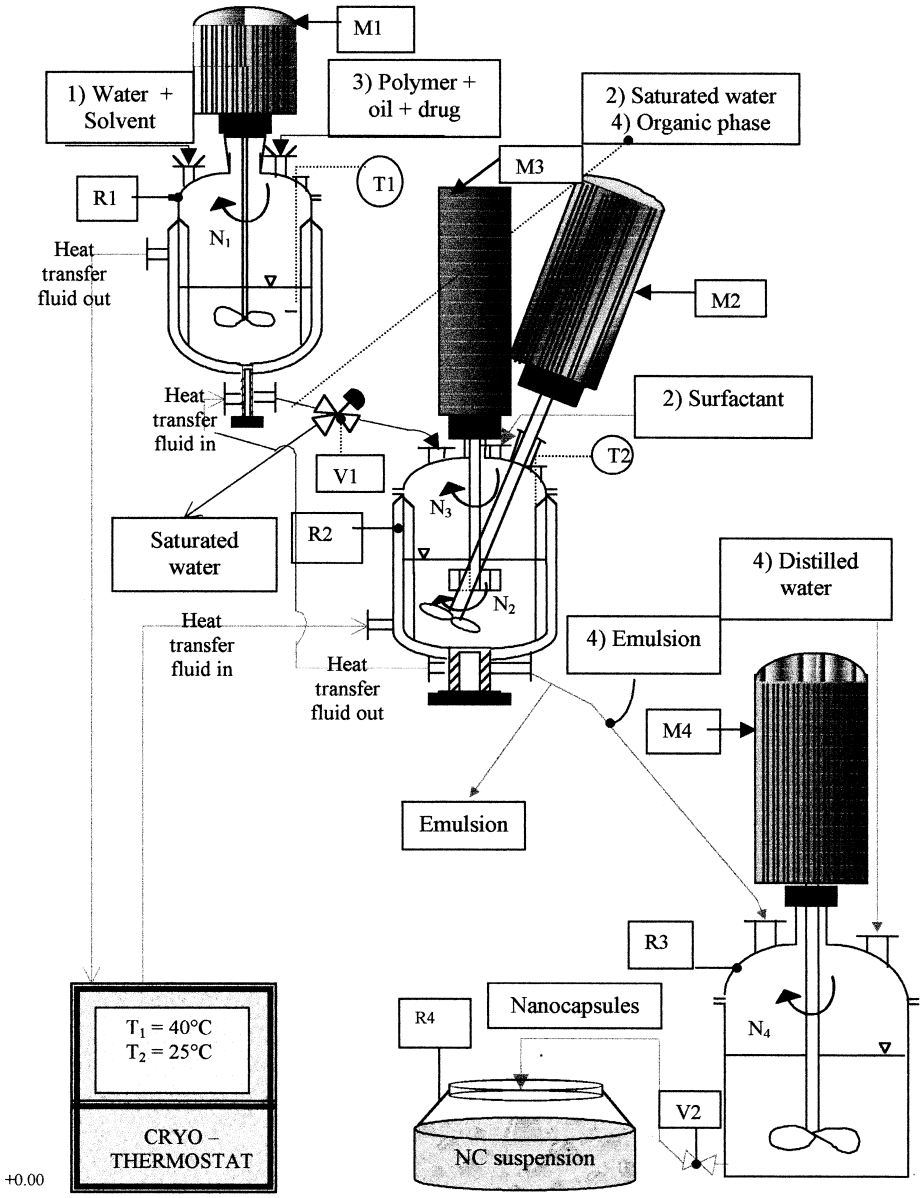

Figure 2. Plant layout: M1, M2, M4, propeller mixer; M3, Ultra-Turrax T50, Ika Labotechnik; N1, N2, N4, propeller rotational speed (rpm); N3, Ultra-Turrax T50 rotational speed (rpm); R1, R2, stirred tank reactors (STRs) equipped with a jacket for the heat transfer fluid circulation, capacities of 2.5 and 2 L, respectively; R3, stirred tank reactor, 6 L capacity; R4, storage tank, 5 L capacity; T1, T2, alcohol thermometers; V1, manual three-way valve; V2, manual drain valve.

For image processing, the morphological examination of NC was performed using a transmission electron microscope (TEM; Philips CM 120) following negative staining with phosphotungstic acid solution (Fig. 3). The sample preparation involves that the suspension of NC is shadowed with collodium coke on a copper grill (200 mesh + 74 μm); after 5 min of evaporation, the grill is covered by the colorant (APT, phosphotungstic acid) and dried on absorbed paper.

Scanning electron microscopy (SEM; Hitachi 5800 with field emission gun) was also used (Fig. 4);

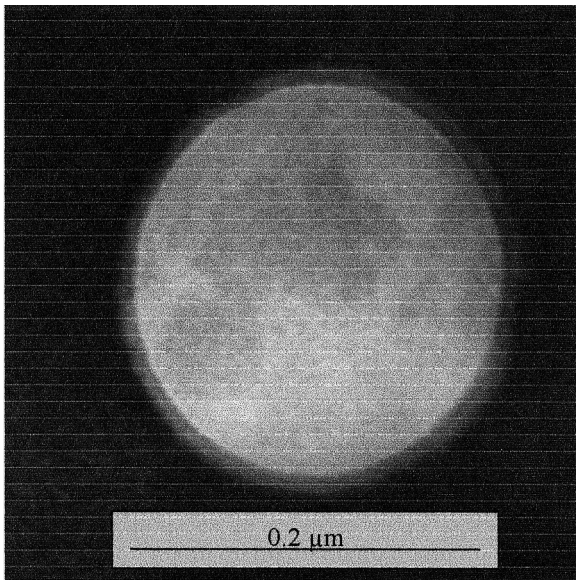

Figure 3. Transmission electron microscopy picture of a nanocapsule.

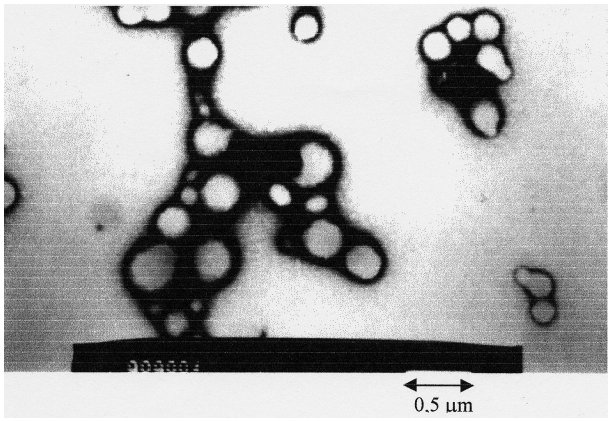

Figure 4. Scanning electron microscopy picture of a nanocapsule.

the sample was frozen by nitrogen liquid, then dried under vacuum (17 Pa) and shadowed with a gold-palladium layer.

RESULTS

We investigated the influence of some operating parameters on the process to control and to optimize the NC formation. The agitation power and duration for the emulsion preparation, the polymer nature and the concentration in the organic phase, the dilution, and the concentration of the suspension were the main parameters studied.

Table 2

Comparison Between Laboratory and Pilot Scale

NC Characterization	Laboratory Scale	Pilot Scale
Final volume	10 ml	2 L
Dry mass fraction	1	32
Emulsion mean diameter (nm)	700 ± 100	500 ± 100
NC mean diameter (nm)	$\sim 500 \pm 250$	$\sim 300 \pm 240$
Drug encapsulation (%)	80 ± 10	80 ± 10

NC, nanocapsule.

Most of the experiments were carried out in the following "standard" conditions: The organic phase (0.65 L) was ethyl acetate saturated with water and containing one of the polymers (PCL or Eudragit E100, 10 $g \cdot L^{-1}$) and the oil (25 $g \cdot L^{-1}$). The two polymers produced the same mean size of particles under the same experimental conditions. Therefore, we decided to carry out most of the experiments with PCL. The aqueous phase (1.3 L) was water saturated with solvent and containing PVAL (25 $g \cdot L^{-1}$). The emulsion was diluted with 3.5 L of distilled water. Variations from these conditions are detailed in each figure and table.

The NCs obtained during the work with the pilot plant were characterized with TEM and SEM (Figs. 3 and 4). Figure 3 shows that the particles are core shells (dark shadow around the particle), and Fig. 4 shows that the surface of the shell does not present any cracks or defects. The mean particle size detected by Coulter analyzer was 0.5 μm. A comparison between the NCs obtained with laboratory and pilot scales is presented in Table 2.

Agitation During Emulsion Preparation (Turbine Rotational Speed and Agitation Time)

Some authors (7) have found that an increase in stirring intensity results in a decay of the emulsion droplet size. In many cases, the variation is linear in a log-log scale. The drop size decreases more rapidly when the apparatus makes use of more turbulent mixing. Quintanar-Guerrero et al. (8) found a decrease of the NP mean size and polydispersity correlated with an increase of the stirring rate.

In our experiments, as shown in the plant layout (Fig. 2), we had to couple a rotor-stator with a classical impeller unit to promote the fluid homogenization inside the reactor R2. If the rotor-stator mixer is more efficient for producing small emulsion droplets than a classical impeller, it does not present enough pumping capacity. Its coupling with the homogenization turbine induces not only a size decay for the emulsion, but also a more uniform size distribution. The same considerations are stated about the agitation duration: Decrease in size and smaller polydispersity is achieved by increasing the agitation time.

Thus, as expected, the emulsion and polydispersity size decrease when the turbine rotational speed N and the agitation time t are increased. These results are shown in Tables 3 and 4 and are plotted in the diagrams of Figs. 5 and 6.

Formulation

Quintanar-Guerrero et al. (5) found that the NP formation is highly dependent on the polymer concentration in the internal phase. The same trend was observed with PCL in our work: A diminution in size was observed with a decrease in polymer concentration due to the diminution of viscosity. This was verified in both laboratory and pilot scales, as shown in Table 5 and Fig. 7.

Table 3

Impeller Rotational Speed

Sample	Speed and Type of Mixer (rpm)		ϕ Emulsion (μm)
	Propeller	Turrax	
1	2000		2.137 ± 0.799
2		5200	0.951 ± 0.443
3	790	8800	0.646 ± 0.269
4	600	10000	0.364 ± 0.120

Preparation conditions: polymer = PCL; concentration 10 $g \cdot L^{-1}$; 10 min agitation.

Table 4

Agitation Time

Sample	Agitation Time (min)	ϕ Emulsion (μm)
1	20	0.408 ± 0.126
2	10	0.646 ± 0.269

Preparation conditions: polymer = PCL; concentration 10 $g \cdot L^{-1}$. Agitation: propeller 790 rpm + Turrax 8800 rpm.

Agitation During the Dilution Step

We did not notice any influence of the stirring speed on the NC size during the dilution step (see Table 6 and Fig. 8). We conclude that the agitation during this step has to be sufficient to homogenize the mixture: With no agitation (curve 4 in Fig. 8), suspension homogeneity is no longer present, and

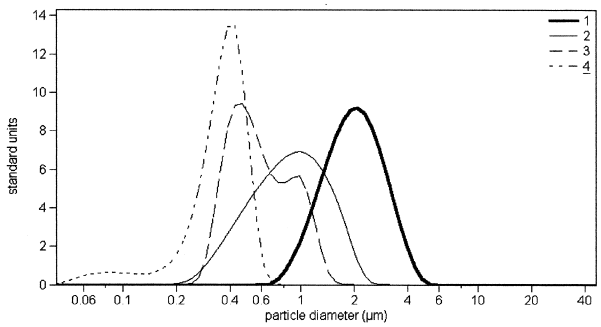

Figure 5. Influence of impeller rotational speed on emulsion size for PCL polymer at a concentration of 10 $g \cdot L^{-1}$ and 10 min agitation: 1, propeller at 2000 rpm; 2, Turrax at 5200 rpm; 3, propeller at 790 rpm plus Turrax at 8800 rpm; 4, propeller at 600 rpm plus Turrax at 10,000 rpm.

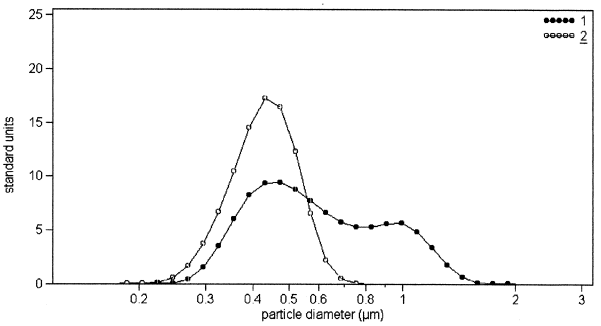

Figure 6. Influence of agitation time on emulsion size for PCL polymer at 10 $g \cdot L^{-1}$ and agitation by a propeller at 790 rpm plus the Turrax at 8800 rpm: 1, agitation for 10 min; 2, agitation for 20 min.

Table 5

Reagent Concentration (Laboratory and Pilot)

Sample (scale)	Polymer ($w_{polymer}/v_{solvent}$)	ϕ NC (μm)
1 (pilot)	7.7	0.284 ± 0.146
2 (pilot)	10	0.370 ± 0.070
3 (pilot)	20	0.761 ± 0.337
4 (laboratory)	10	0.364 ± 0.127
5 (laboratory)	20	0.605 ± 0.250
6 (laboratory)	30	0.820 ± 0.360
7 (laboratory)	40	1.400 ± 0.660

Preparation conditions for pilot scale: polymer = PCL; 20 min agitation; agitation with propeller at 790 rpm + Turrax at 8800 rpm. Preparation conditions for laboratory scale: polymer = PCL; 5 min agitation; Turrax 8000 rpm.
NC, nanocapsule.

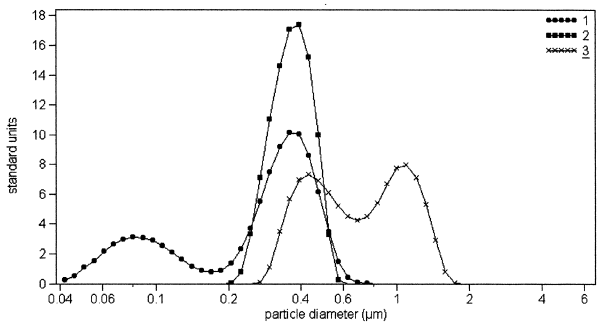

Figure 7. Influence of polymer concentration on nanocapsule size for PCL polymer agitated for 20 min by the propeller at 790 rpm plus the Turrax at 8800 rpm.

Table 6

Impeller Rotational Speed During Dilution (Pilot)

Sample	Velocity Propeller (rpm)	ϕ Emulsion (μm)	ϕ Nanocapsules (μm)
1	800	0.580 ± 0.485	0.299 ± 0.132
2	600	0.397 ± 0.093	0.234 ± 0.118
3	300	0.580 ± 0.485	0.281 ± 0.137
4	0	0.397 ± 0.093	0.178 ± 0.109 (2 population)

Preparation conditions: polymer = Eudragit E100; concentration 10 $g \cdot L^{-1}$, 10 min agitation for emulsion; propeller at 790 rpm + Turrax at 8800 rpm.

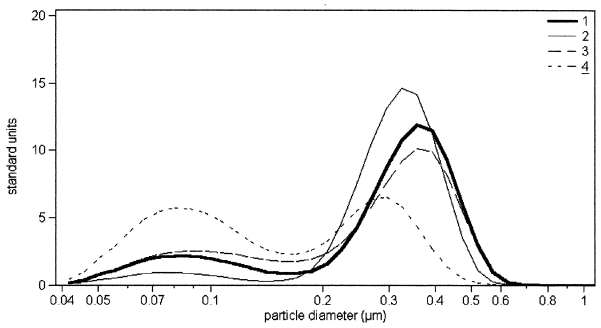

Figure 8. Impeller rotational speed influence on nanocapsule size during dilution for Eudragit E100 at a concentration of 10 $g \cdot L^{-1}$ with the emulsion agitated for 10 min by the propeller at 790 rpm plus the Turrax at 8800 rpm.

the polydispersity increases. Therefore, the NC formation is not governed by the macroscopic mixing, but rather by the solvent migration occurring at the microscopic level at the liquid-liquid interface.

In the same way, the agitation time t during the dilution step does not influence the NC size. The system needs a certain time to achieve stability, as explained in the next section.

Solvent Evaporation

The concentration step involves solvent evaporation and partial water elimination. A settling period after the NC formation is needed before evaporation. This period allows the diffusion of the remaining solvent from the NC core shells to the liquid bulk. If evaporation is performed before this rest time, which was evaluated at 1 h, the NC membrane risks being destroyed. Otherwise, after the rest time, the solvent and water evaporation rates do not affect the NC structure (Fig. 9).

Encapsulation Tests

Preliminary tests were performed for drug encapsulation with indomethacin as the active agent. It was dissolved in the organic phase before the emulsion formation; the other preparation conditions were unchanged. The mean size of NC was not affected by the incorporation of the drug in the particles. The drug encapsulated ratio was analyzed by separating the NCs from suspension (by ultracentrifugation), collecting the NCs, and dissolving them in an organic solvent (generally,

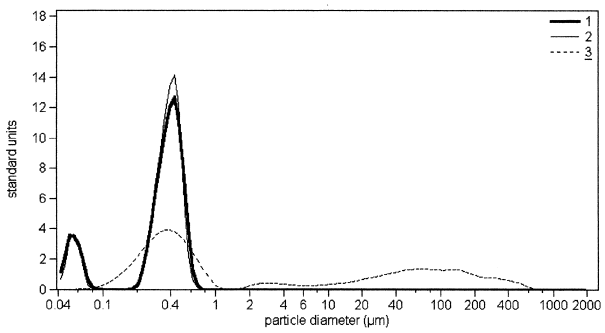

Figure 9. Influence of evaporation on nanocapsule size for PCL polymer at a concentration of 10 $g \cdot L^{-1}$ with the emulsion propeller at 790 rpm plus the Turrax at 8800 rpm; agitation during dilution was 600 rpm; rest time was 1 h: 1, nanocapsules before evaporation; 2, nanocapsules after evaporation; 3, nanocapsules evaporated immediately after dilution (without rest time).

acetonitrile). The drug content was then assayed by UV spectrophotometry (Varian®, Cary 50). The results showed that 80% (±10%) of the drug initially introduced was trapped in the NCs. The same results were obtained at the laboratory and the pilot scales.

SCALE-UP

We used the results obtained at the laboratory scale (0.06 L) to design and assemble the pilot plant (2 L). This plant was built using classical chemical engineering equipment; the geometry of the reactor is standard, so it can be extrapolated by geometric similitude. It is the same for the agitation systems used; the propellers and the rotor-stator unit are standard and well defined by the manufacturers. They are adapted to an extrapolation at a larger scale (Rapport Mixel®, Hydraulique: Notions Théoriques de Base).

As we have well-characterized tools, we can easily design an industrial plant (1000 L) according to the following method based on the similitude. Different parameters can be kept constant: the Reynolds number, the power consumption per volume unit, or the agitation speed (9–11). All the calculations refer to the emulsion preparation, so the reactor involved is the so-called R2 with its dispersion unit. We consider an extrapolation from 2 L to 1000 L (Table 7).

Table 7

Scale-Up

	Pilot Plant	Industrial Plant
V (l) = capacity	2	1000
T (m) = diameter of reactor	0.136	1.08
D (m) = diameter of dispersion unit	0.04	0.3
N (rps) = dispersion unit velocity	147 (8800 rpm)	?
D' (m) = diameter of impeller	0.05	0.4
N' (rps) = impeller velocity	13 (790 rpm)	?

The calculation gives the order of magnitude of the vessel dimensions and operating conditions of the industrial plant. The extrapolation method was based on the similitude: for the rotor-stator, ND = constant (where N is the rotational speed [rpm] and D is the diameter [m]). Here, we considered that the peripheral speed of the rotor (represented by ND) is the most important parameter because it is directly linked with the shear forces responsible for the decrease of the droplet size. So, this parameter has to be kept constant for extrapolation to ensure the control of the size distribution of the emulsion whatever the production capacity. This value was 5.9 $m \cdot s^{-1}$ in our case.

For the second impeller unit, P/V = constant $\propto N^3D^2$ (where P is the power transmitted by the impeller shaft [W] and V is the capacity [L]). The power transmitted by the impeller to the liquid is calculated by the classical relation (9–11): $P = \rho N_p N_3 D_5$, with N_p being a characteristic parameter of the impeller, the power number (1.5 in our case). The calculation leads to 1.09 W in the 2-L reactor, so the power per volume unit is about 540 $W \cdot m^{-3}$.

By this calculation based on similitude and considering $ND_{\text{rotor-stator}} = 5.9\ m \cdot s^{-1}$ = constant and $P/V_{\text{impeller}} = 540\ W \cdot m^{-3}$ = constant, we obtained the subsequent results:

$$1100\ \text{rpm} < N_{\text{rotor-stator industry}} < 1200\ \text{rpm}$$

$$150\ \text{rpm} < N_{\text{impeller industry}} < 300\ \text{rpm}$$

CONCLUSION

This study presents the scale-up of a new process developed for producing colloidal polymeric drug vectors by the so-called emulsification-diffusion technique patented by Quintanar et al. (12).

The aim was to check if classical tools for the pharmaceutical industry can be used to perform NC preparation. Thus, the scale-up from the laboratory (0.06 L) to the pilot plant (2 L) allowed assembly of the pilot plant. The equipment used in the pilot plant was adequate for industrial scale-up purposes (1000 L) according to chemical engineering techniques.

The pilot plant was used successfully to prepare NCs by the emulsification-diffusion technique. The scale-up from the laboratory to the pilot plant allowed obtaining NCs in an efficient and reproducible manner. The mean particle size was 0.3 μm using PCL and 0.24 μm using Eudragit E100 as the polymer. Almost all the dispersions were stable for at least 5 months of storage at room temperature.

To achieve our aim (that is, the control of NC size), different parameters of the emulsion preparation were detected due to the NC size dependence on emulsion size. In fact, as shown in the results, the emulsion presenting the smallest size allowed the achievement of the smallest NC size (Table 6).

The mean parameters involved and their influence on the emulsion particle size are summarized below:

Decrease of emulsion size by increasing impeller rotational speed
Decrease of emulsion size by increasing agitation time
Decrease of emulsion size with diminution of polymer concentration

The polymer concentration is a very important parameter, closely associated with the capsule wall thickness and, therefore, with the drug delivery kinetics.

Concerning the dilution step, we concluded that agitation time and stirring speed do not influence the NC size.

Finally, we confirmed that evaporation under reduced pressure is a good method to concentrate the NC suspension.

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