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FORMULATION DEVELOPMENT AND CHARACTERIZATION OF NANOPARTICLES LOADED WITH ANTI DIABETIC DRUGS AND SCALE UP THROUGH VARIOUS EFFICIENT TECHNIQUES

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ABSTRACT: The lack of information related to the scaling-up of technologies used for preparing polymeric nanoparticles (NP) might hinder the introduction of these colloidal carriers into the pharmaceutical market. In the present study, the scale-up of Repaglinide-loaded NP produced by three manufacturing processes – salting-out, emulsification–diffusion and nanoprecipitation – was assessed at pilot-scale by increasing 20-fold the laboratory- batch volume from 60 ml to 1.5 l. Drug Coat L 100-55and poly (vinyl alcohol) (PVAL) were used as polymer and emulsifying agent, respectively. The influence of the hydrodynamic conditions on the NP characteristics such as mean size, drug content, residual PVAL and morphology was also investigated. At pilot-scale, stirring rates of 790–2000 rpm lead to NP mean sizes ranging from 557 to 174 nm for salting-out and from 562 to 230 nm for emulsification–diffusion. An increase in the stirring rate enhances the droplet break-up phenomenon which leads to the formation of finer emulsion droplets and thus smaller NP. Moreover, the influence of the stirring rate on the mean size of NP can be predicted using a model based on a simple power law. The continuous method used for nanoprecipitation scale-up allows production of NP in a reproducible way over a relatively short time. Finally, for the three methods, NP characteristics were reproduced well at both scales. However, the scale-up process induced a slight reduction in the size and drug loading of NP.

Introduction:

The introduction of a new product on the market is preceded by different stages of research and development. During the course of its development a series of progressive refinements in the formulation, manufacturing processes and product presentation take place. The scale-up phase includes the integration of procedures, as well as the transfer of technology, for realizing the large-scale manufacture of a given product. This stage of development is crucial because, very often, many of the process limitations that are not apparent on the small scale become significant on a larger scale, and may even lead to the failure of translating a unit to industrial dimensions. In practice, the transition from a laboratory system to a plant system is not direct. The product is commonly prepared on intermediate scales, larger than the initial development studies but smaller than the industrial manufacturing. Basically, the idea is to simulate production as much as possible and to optimize the operating parameters before large-volume work is performed. A scale-up procedure based on a well-prepared technical transfer will assure product quality, overall economy and timely achievement of market readiness.

Although polymeric nanoparticles (NP) have been recognized as one of the most promising colloidal drug delivery systems, their introduction into the pharmaceutical market is likely to be hindered because of a lack of information concerning the scale-up of technologies used for their preparation. The existing information related to this topic is restricted to very few studies (Colombo et al., 2001; Galan Valdivia et al., 1998; De Labouret et al., 1995). Obviously, with such limited information, efficient scale-up and successful operation at industrial scale is almost impossible. Therefore, in the present study, the scale-up of three manufacturing processes of NP – salting-out, emulsification–diffusion and nanoprecipitation – was assessed by increasing 20-fold the volume of the laboratory batches. The influence of the scale-up, particularly the hydrodynamic parameters, on the NP characteristics such as mean size, drug loading, residual surfactant, and morphology was also investigated. Drug Coat L 100-55, poly (vinyl alcohol) (PVAL), and Repaglinide were used as polymer, emulsifier agent and model drug, respectively. The optimized formulation variables and process parameters for preparing Repaglinide - loaded NP at laboratory scale were selected considering the findings reported in previous studies (Galindo- Rodr´ıguez et al., 2004, in press).

Materials and methods

Materials

Repaglinide was a gift from Cadila Pharma (Dholka, Ahmadabad). Poly (vinyl alcohol), with a molecular weight of 26,000 and a hydrolysis degree of 88% (Mowiol® 4-88), was kindly supplied by J.B.C.P.L (Panoli, Ankleshwar). Methacrylic acid copolymer Type C USP/NF (Drug Coat L100-55) was a gift from Colorcoan, mumbai). All other chemicals used were of reagent grade

Procedures for nanoparticle production.

Nanoparticles were prepared according to standard procedures of the salting-out (Bindschaedler et al., 1988), emulsification–diffusion (Leroux et al., 1995) and nanoprecipitation (Fessi et al., 1989) methods.

Modifications of these general procedures (Fig. 1) will be detailed in the Section 3. In all cases, scale-up was achieved by increasing 20-fold the materials used for the lab-scale batches. Table 1 presents the exact composition of the aqueous and organic phases used for the three methods.

Salting-out

For the lab-scale, 50 g of an aqueous solution were added under mechanical stirring (Remi mechanical stirrer, Remi india, mumbai) to 30 g of an organic phase. Agitation was maintained at the required stirring rate for 15 min. After emulsification, 50 g of pure water were added to induce the diffusion of the organic solvent into the external phase and the formation of NP. The dimensions of the agitation systems used for the emulsification step are shown in Table 2. For the pilot-scale experiments, 1000 g of aqueous phase, 600 g of organic phase and 1000 g of pure water were used.

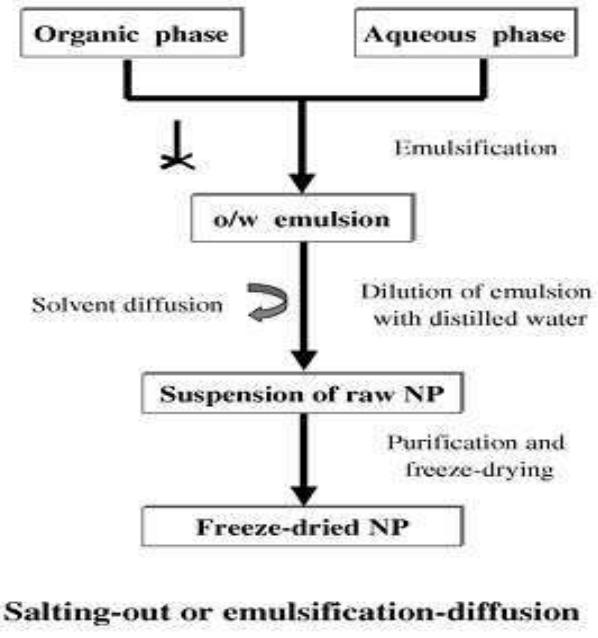

Fig. 1. General procedures of nanoparticle preparation

Emulsification–diffusion

For the lab-scale, 40 g of an aqueous solution were added under stirring to 21g of an organic solution. The resulting o/w emulsion was stirred continuously for 15 min. Then, 660 g of water were introduced in order to allow the diffusion of the organic solvent into the water, leading to NP formation. Emulsification was performed using the same agitation system as that for salting-out. Scale-up for this method was carried out using 800 g of aqueous phase, 420 g of organic phase and 13,200 g of pure water.

Table 1 : Compositions (%, w/w) of the aqueous and organic phases used in the salting-out , emulsification–diffusion and nanoprecipitation methods

Formulation	Salting out	Emulsification–diffusion	Nanoprecipitation
Organic phase			
Drug Coat L 100 - 55	9	14.3	1.44
Repaglinide	1	1.4	0.14
Organic solvent	90[a]	84.3[b]	98.42[a]
Aqueous Phase			
Mowiol® 4-88	9	12	0.8
$MgCl_2 \cdot 6H_2O$	34	-	-
HCl concentration q.s. (pH)	1.5	1.5	-
Distilled water	57	88	50

[a] Acetone was used for salting-out and nanoprecipitation

[b] Benzyl alcohol was used for emulsification–diffusion

Pilot-scale production of the emulsion-based methods.

The equipment used for pilot-scale production was composed of three double-jacketed reactors which can be connected depending on the requirements of each fabrication process. The characteristics (dimensions) of the main reactor, in which the emulsification step is performed (R-2 in Fig. 2), are presented in Table 2. This reactor had a total capacity of 2 l and was equipped with four baffles. Its content was stirred (Eurostarpower control-visc stirrer, IKA-WERKE, Staufen, Germany) with a 45◦ pitched blade turbine. The characteristics of the other reactors are mentioned in Fig. 2. The experimental set-up for the salting-out (Fig. 2(A)) and emulsification diffusion (Fig. 2(B)) methods will also be described in the discussion of results. The flow of the products from one reactor to another was enabled by gravity. Stirring rate ($790 \leq N \leq 2000$ rpm), in a range generally corresponding to a turbulent flow regime, was the parameter evaluated for the two methods at both scales. The flow regime for each emulsified system was determined considering the dimensionless Reynolds number (Re) which was calculated from Eq. (1):

$$Re = \frac{\rho c N D^2}{\eta c}$$

where N is the stirring rate (s−1), D is the impeller diameter (m), ρc is the density of the continuous phase (kg/m3) and ηc is the viscosity of the continuous phase (Pa s). Experimental values of N, D, ρc and ηc are reported in Tables 2 and 3. As shown in Table 4, most of the emulsions were prepared under a turbulent flow regime ($Re \geq 104$). Only a few emulsions produced at lab-scale for the salting-out method (from 790 to 1250 rpm) had a Re that was slightly lower than 104.However, because of the negligible difference, the few regimens can be considered to be turbulent in nature.

Table 2

Main characteristics of the laboratory and pilot agitation systems used in the emulsification step of the salting-out and emulsification–diffusion methods

Characteristics	Lab scale	Pilot scale
Reactor capacity [a] (l)	0.2	2
Reactor internal Diameter (m)	0.07	0.15
Volume of emulsion (l)		
Salting out	0.076	1.528
Emulsification–diffusion	0.054	1.173
Impeller diameterb (m)	0.04	0.08
Number of baffles	-	4
Stirring rate (rpm)	790–2000	790–2000

[a] This corresponds to R-2 in Fig. 2.
[b] 45◦ pitched blade turbine.

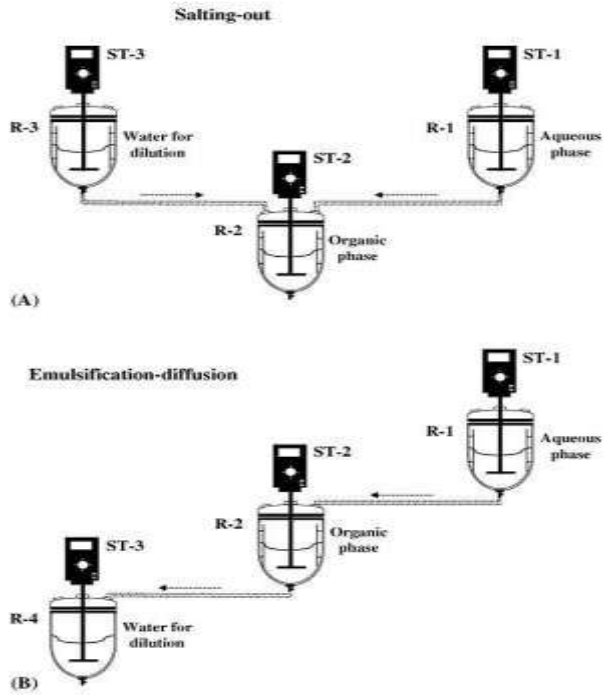

Fig. 2. Set-up for the scaling-up of the two emulsion-based methods. Abbreviations: ST-1, ST-2 and ST-3, stirrers (Eurostar power control-visc stirrer, IKA-WERKE, Staufen, Germany); R-1, 2.5 l reactor; R-2, 2.0 l reactor; R-3, 2 l reactor; R-4, 15 l reactor; all stirred reactors were equipped with four baffles and agitated with a 45◦ pitched blade turbine; only for R-4 baffles were excluded.

Table 3: Physicochemical properties[a] of the fluids involved in the preparation of nanoparticles by the salting-out and emulsification–diffusion methods

Property	Salting out			Emulsification Diffusion		
	Aqueous phase	Organic phase	Emulsion	Aqueous phase	Organic phase	Emulsion
Viscosity (Pa s)[b]	0.119	0.012	0.075	0.039	2.177	0.545
Density (g/ml)[c]	1.161	0.812	1.047	1.24	1.066	1.040
Interfacial tension (mN/m)[d]	--	--	1.34 *	--	--	2.44 *
Surface tension (mN/m)[d]	44.8	24.8	--	42.6	39.8	--

[a] All measurements were performed in triplicate at 25 °C.
[b] Viscometer Contraves model Rheomat 15 T, Zurich, Switzerland.
[c] Pycnometer, 10.08 ml capacity.
[d] Tensiometer Kr uss model K12, Kr uss GmbH, Hamburg, Germany.
[e] Interfacial tension between the aqueous and organic phases.

Nanoprecipitation

For the lab-scale (Fig. 1), the organic solution (25 ml) was added to the aqueous phase (50 ml) containing the stabilizing agent and stirred magnetically. The solvent was then evaporated under reduced pressure. For the pilot-scale, the required volumes of the organic and aqueous phases were 500 and 1000 ml, respectively.

Pilot-scale production of the nanoprecipitation method

The scale-up for this technique was performed using a continuous method which has been well characterized and optimised previously (Brianc¸on et al., 1999). A schematic diagram of the experimental set-up is shown in Fig. 3.

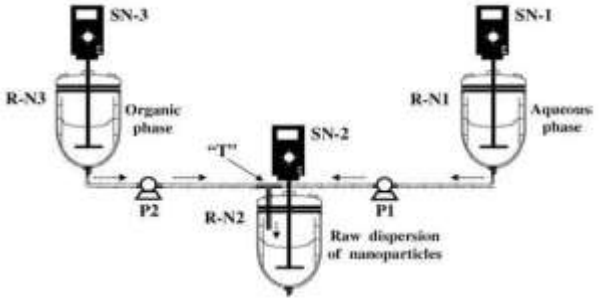

Fig. 3. Set-up for the scaling-up of the nanoprecipitation method. Abbreviations: SN-1, SN-2 and SN-3, stirrers (RW 20DZM.n stirrer, IKAL aborrechnik, Staufen, Germany); R-N1, R-N2 and R-N3, 2.5 l reactors equipped with four baffles and an axial impeller; P1 and P2, peristaltic pumps; T, "Tee mixer".

This consists of three reactors (R-N1, R-N2, R-N3) each equipped with an axial impeller. The R-N1 and R-N3 reservoirs contain the aqueous and organic phases, respectively. Both phases are continuously supplied by independent peristaltic pumps (P1and P2). The interesting point of this continuous system is a "Tee mixer" which serves to mix the two phases. In fact, when both phases come into contact in the central part of the "Tee

mixer", they diffuse into each other forming immediately the NP. The raw NP dispersion is finally received in the main reactor (R-N2) and maintained under a gentle agitation.

Purification of nanoparticles

Following the preparation stage, purification of NP was carried out by centrifugation (Centrifuge model Avanti 30, Beckman Instruments, California, U.S.A.) for three different batches prepared at lab- and pilot-scales. Briefly, a sample of raw NP dispersion (60 g) was first separated from free surfactant and solvent at 25,000×g/30 min. Nanoparticles were then washed twice using 30 ml of water adjusted to pH 3.0 with concentrated HCl (20,000×g/20 min) and finally with deionized water (20,000×g/20 min); 100 min were necessary for purification of one batch of NP. After recovery, the purified NP were freeze-dried (LSL Secfroid, model Lyolab BII, Switzerland).

Table 4:-Calculated Reynolds number (Re) for emulsified systems formed during NP preparation by the salting-out and emulsification–diffusion methods

Stirring rate (rpm)	$Re_{salting\text{-}out}$ ($\times 10^{-4}$)		$Re_{emulsification\text{–}diffusion}$ ($\times 10^{-4}$)	
	Lab-scale	Pilot-scale	Lab-scale	Pilot-scale
2000	1.3	2.6	3.5	7.0
1750	1.1	2.3	3.1	6.1
1500	1.0	2.0	2.6	5.3
1250	0.8	1.6	2.2	4.4
1000	0.7	1.3	1.8	3.5
790	0.5	1.0	1.4	2.8

[a]Re was calculated from Eq. (1) using experimental data from Tables 2 and 3.

Characterization of nanoparticles

Analysis of nanoparticle mean size

The mean size and polydispersity of raw NP dispersions were assessed by photon correlation spectroscopy (Zetasizer 3000®, Malvern Instruments, Worcestershire, U.K.).With respect to the polydispersity index (P.I.), which ranges from 0 to 1, a higher value corresponds to a less homogeneous NP size distribution.

Drug loading

Samples of freeze-dried NP were accurately weighed and dissolved in 0.1N NaOH. The Repaglinide content was determined spectrophotometrically at λmax = 243 nm (Shimadzu 1201 UV-visible spectrophotometer). Three samples were examined for three different batches of each NP formulation.

Residual PVAL

Nanoparticles were assayed for residual PVAL using a method that involves the formation of a stable complex of PVAL with iodine in presence of boric acid (All´emann et al., 1993). First, freeze-dried NP were dissolved in 0.1M NaOH. Subsequently, Drug Coat L 100-55 was precipitated by the addition of 0.1M HCl and the suspension centrifuged. Then, an aliquot of supernatant was treated with 7.5 ml of boric acid solution (4.0%, w/v) and 1.5 ml of iodine solution (1.27% iodine and 2.50% potassium iodide in distilled water, w/v), and the volume adjusted to 25.0 ml with water. Finally, the absorbance was measured at 644 nm.

Scanning electron microscopy

Morphological examination of NP was performed using a scanning electron microscope (model JEOL JSM- 6400, Jeol Ltd., Japan). Samples of dried NP were dispersed in water, and then drops of the dispersion were placed on metallic studs and coated with gold.

Characterization of the fluids involved in the emulsion-based methods Viscosity

The viscosity of the aqueous phases, organic phases and emulsions, prepared for the salting-out and emulsification– diffusion methods, was determined at 25 ◦C using a cone-plate system (Oswald viscometer). Each determination was made in triplicate.

Surface and interfacial tensions

The plate method was used for surface and interfacial tension determinations (Digital Tensiometer K12, Kr¨uss, Hamburg, Germany). Each measurement was made in triplicate at 25 0C.

Density

Density measurements were carried out using a picnometer. Each determination was performed in triplicate at 25 ◦C.

Results and discussion

Preparation of nanoparticles by the emulsion-based methods

During NP preparation using the emulsion-based methods, such as salting-out and emulsification–diffusion, a nanoemulsion is formed due to dispersion of an organic phase into an aqueous phase by shear mixing. After emulsification, water is added to the emulsion in order to remove the solvent from the droplets and induce NP formation. At that point, it has been established that NP features, such as size, are determined by the characteristics of the precursor nanoemulsion (Galindo-Rodr´ıguez et al., 2004; Lemarchand et al., 2003). This fact is of great importance because the final droplet size of a liquid-liquid dispersion in agitated vessels depends on parameters such as the physicochemical characteristics of the two phases (e.g. viscosity, interfacial tension, stabilizer concentration), the preparation conditions of the emulsion (e.g.temperature, addition order of the components) and the agitation system (e.g. shear rate, design of the stirrer and containing vessel). In particular, because one of the major problems in the transition from lab-scale to pilot-scale is to provide similar hydrodynamic conditions, the first part of this study focused on determining the effect of stirring rate on the NP mean size.

Lab-scale production

Nanoparticles were produced according to salting-out and emulsification procedures. Although both preparation techniques are quite similar (Fig. 1), the dilution–diffusion step is slightly different. Whilst pure water is added to the emulsion for salting-out, the emulsion is incorporated into water for emulsification–diffusion. In the first case, this is technically possible because the reactor containing the emulsion is large enough to accommodate the water. In contrast, for the second case, because a much larger volume of water is needed for dilution (600 ml) and the reactor capacity is limited to 200 ml, the emulsion has to be transferred to a larger reactor which contains the pure water for dilution. As illustrated in Fig. 4, NP mean size decreases on increasing the stirring rate. For salting-out, mean sizes of NP from 719 to 279 nm were obtained when the stirring rate was varied from 790 to 2000 rpm. In emulsification–diffusion, for the same range of stirring rate, NP mean sizes ranged from 421 to 300 nm. This can be explained considering that, in a stirred vessel, the mean droplet size of an emulsion system is governed by the balance between the break-up and coalescence of droplets. These phenomena occur simultaneously during emulsification, so their relative kinetics determine the final droplet size.

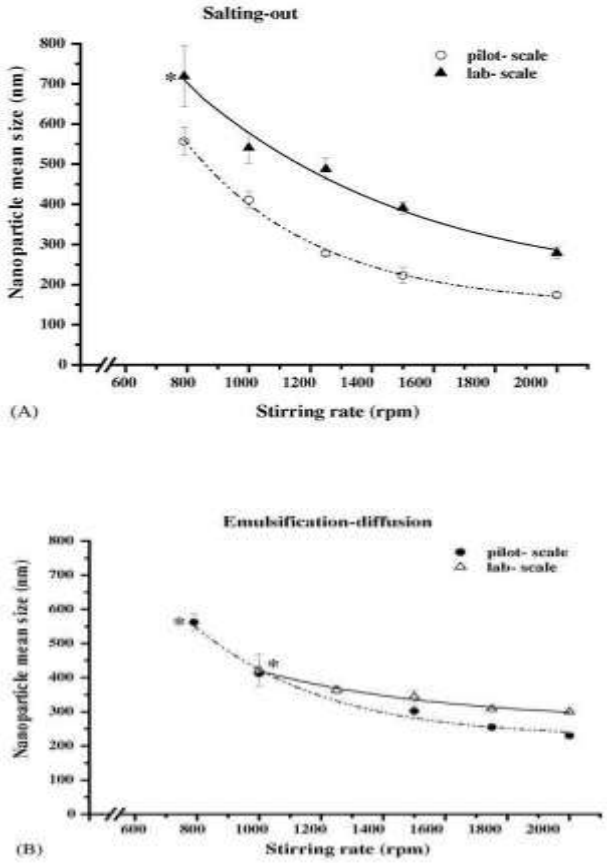

Fig. 4. Influence of stirring rate on the mean size of nanoparticles prepared by salting-out and emulsification–diffusion at the laboratory and pilot scales. An abundant presence of particles larger than 1 μm was found in batches marked with * (mean ± S.D., n = 3).

The maximum diameter of the emulsion droplets is linked to the break-up mechanism whereas the minimum diameter depends on the coalescence phenomenon (Baldyga et al.,2001). Likewise, the breakage forces depend on the power input per unit mass, which increases by increasing the stirring speed. Therefore, the droplet break- up process increases with the stirring speed leading to formation of finer emulsion droplets, and thus smaller NP. On the other hand, for a given stirring rate, particle size was generally reproducible. Very similar results were obtained from three separate batches as attested by the low standard deviation values (S.D. in Fig. 4). This

also shows that the mixing efficiency was acceptable. However, at stirring rates ≤790 rpm for salting-out and ≤1000 rpm for emulsification diffusion, raw NP dispersions were heterogeneous in their size distribution and exhibited the presence of particles larger than 1μm (batches marked with [*] in Fig. 4). These findings revealed that break- up efficiency decreased, while the coalescence process was favoured, when the stirring rate was reduced.

Pilot-scale production

Scale-up production for emulsion-based methods was carried out by increasing 20-fold the volume of the laboratory batches. For salting-out, the set-up is illustrated in Fig. 2(A).

Geometric similarities of the agitation systems used at laband pilot-scales for the emulsification step (Table 2) were maintained as much as possible in order to obtain a similar fluid motion at both scales. Briefly, the organic phase was first introduced in R-2, and the aqueous phase was then added from R-1 by gravity. After 30 min of emulsification, the pure water contained in R-3 was incorporated. Agitation was maintained for 5 min. For emulsification–diffusion, because of the large volume of water used for the dilution step, the configuration of the reactors previously used for salting-out was changed. The third reactor was substituted with another (R-4) of a larger capacity (15 l). The experimental set-up used for this technique is shown in Fig. 2(B). Under these conditions, it was possible to reproduce the lab-scale procedure. The aqueous phase contained in R-1 was added to R-2 containing the organic phase. Stirring was maintained for 30 min. Afterwards, the emulsion was transferred into R-4 which had previously contained the pure water intended for the dilution–diffusion step.

During introduction of the emulsion, R-4 was gently stirred.

As expected, when increasing the agitation rate, the NP mean size clearly shifted to smaller diameters (Fig. 4). The NP mean sizes varied from 557 to 174 nm for salting-out and from 562 to 230 nm for emulsification– diffusion when the stirring rate changed from 790 to 2000 rpm. It was also noted that, for stirring rates ≥1000 rpm reasonably narrow size distributions were obtained which, in turn, revealed that uniform emulsification processes were achieved. However, below 1000 rpm for emulsification–diffusion and 790 rpm for salting-out, the high values of the standard deviation (S.D. in Fig. 4) show that batch-to-batch reproducibility was severely affected, which may be attributed to a heterogeneous dispersion of the organic liquid phase. In those cases, the insufficient energy input supplied by the stirrer as well as the high viscosity of the external phase led to a decrease in emulsification break-up efficiency thus favouring droplet coalescence. This results in heterogeneous size distributions for the individual batches which systematically exhibited the presence of a high number of particles larger than 1 μm (batches marked with [*] in Fig. 4). Obviously, a lack of homogeneity of the individual batches leads to high differences when inter-batch comparisons are made. When comparing the curves obtained at the lab- and pilotscales, it is observed that the patterns are quite similar in the salting-out method and emulsification–diffusion methods (Fig. 4), which indicates that the break-up and coalescence processes were reproduced with the agitation systems employed at both scales.

Theoretical considerations for modeling the emulsion-based methods

In the literature, mathematical models for the formation of NP produced by salting out and emulsion diffusion are not yet established. Because in both processes the size of NP is determined by the size of the precursor nanoemulsion, it is convenient to consider a model for describing the formation of the droplets in the nanoemulsion. Here, we present a classical theory used for modeling dispersed liquid–liquid systems in stirred vessels originating from the chemical engineering field (Baldyga et al., 2001). Although this theory has been developed for emulsions in the micrometric size range, some authors have recently attempted to apply it to the case of nanoemulsion systems (Rivautella et al., 2003). The phenomenon of droplet dispersion has been studied by

many researchers based on the fundamental papers by Kolmogorov (1949) and Hinze (1955). The theory of local isotropic turbulence applicable to a dispersed phase gives a relationship between the maximum stable drop size of the droplets (dmax, m) and the stirring rate (N, s−1), the stirrer diameter (D, m), the interfacial tension (σ, Nm) and the density of the continuous phase (ρc, kg/m3):

$$d_{max} \alpha N^{-6/5} D^{-4/5} \sigma^{3/5} \rho c^{-3/5} \quad (2)$$

Because it is not possible to measure the maximum stable drop size, the dmax is often substituted by the surface- volume mean diameter (Sauter diameter). Likewise, when the Sauter diameter is not known, a mean volume diameter is also accepted. It should be also noted that the Eq. (2) is mainly valid if the hydrodynamic flow regime is turbulent. As discussed in Section 2.2.3, most of the systems analyzed in this study satisfied this condition.

Modeling the effect of the stirring rate on the nanoparticle mean size. From Eq. (2), it is possible to express the evolution of the droplet mean size with the stirring rate as follows:

$$[\rho c]^{-0.6} D^{-0.8} N^{-1.2} \quad (3)$$

$$d_{mean\ drop} = K2 \sigma$$

where K2 is a constant. Moreover, for the case of emulsioned systems prepared at the same scale (where D, ρc, and σ are constant), Eq. (3) can be reduced as following in a logarithmic form:

$$\log d_{mean\ drop} = \log K3 - 1.2 \log N \quad (4)$$

where K3 is a constant. Due to the instability of the nanoemulsion formed during NP preparation by the salting- out and emulsification–diffusion methods, it was not possible to determine the size distribution of the droplets of the emulsion. Nevertheless, one can assume (a) that each emulsion droplet leads to the formation of one nanoparticle, and (b) that the droplets and the particles are non-porous. Experimental evidence of the first assumption was reported in a previous study.

(Galindo-Rodr´ıguez et al., 2004). So, Eq. (4) can be represented as follows:

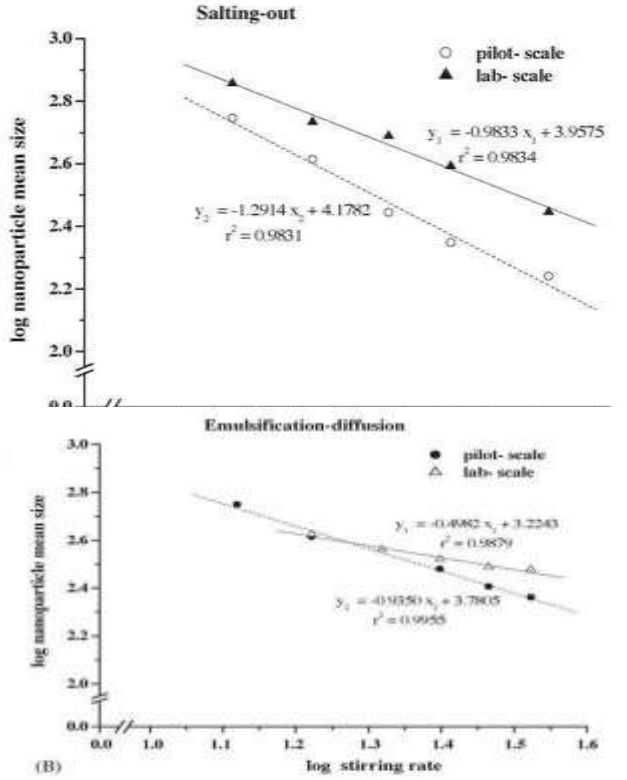

Fig. 5. Logarithmic relationship between the stirring rate and diameter of nanoparticles (Eq. (4)) prepared, at lab- and pilot-scales, by the salting-out and emulsification–diffusion methods. The slope of the curves also corresponds to exponent of N in Eq. (5).

$$d_{mean\ nanoparticle} \alpha d_{mean\ drop} \alpha N^{-1.2} \quad (5)$$

where dmean nanoparticle corresponds to the mean size of nanoparticles.

The logarithmic relationship between the stirring rate and the mean size of nanoparticles obtained by the salting- out and emulsification–diffusion methods is plotted in Fig. 5, at both lab- and pilot-scales. Each series of data was fitted using a linear relationship where the slope (m) of the curves corresponded to the exponent of N in Eq. (5). At pilotscale, the experimental values obtained for salting-out (−1.3) and for emulsification–diffusion (−0.9) were quite close to the theoretical one (−1.2) which indicates that this model can adequately describe the influence of stirring rate on the nanoparticle mean size. Other authors have found a similar behavior for emulsified systems in the micrometer (J´egat and Taverdet, 2000). In contrast, two situations might be observed at lab-scale. While the value of the slope obtained from the salting-out method (−1.0) was considered as correct, the value resulting from emulsification–diffusion (−0.5) showed a significant difference compared to the theoretical value (−1.2). This discrepancy could have multiple origins. For instance,

it is suspected that the stirring configuration was not well-defined at lab-scale (e.g. the absence of baffles). However, since this factor is not sufficient to explain the deviations at lab-scale, other unknown factors have to be investigated in further studies.Based on the pilot-scale results, it might be concluded that the tested model, usually applied to liquid– liquid dispersions sized in the micrometer range and having a low volume fraction of dispersed phase, was suitable for predicting the effect of stirring speed on the NP mean size.

Modeling the effect of scale-up on the mean size of nanoparticles.

The important concept for scale-up in chemical engineering science is the principle of similarity which has been explained widely by Levin, 2002 for pharmaceutical processes. In the case of the emulsions prepared in stirred vessels, the first type of similarity to consider is the geometric one: two systems are geometrically similar when the ratio of the linear dimensions of the small-scale vessel and scale-up vessel is constant. The second type of similarity to take into account is the dynamic one. This is obtained when the specific power input of the stirrer (ε) is kept constant. ε is the ratio of the power (P) imparted by the stirring to the volume (V) of the emulsion. ε can also be expressed in relation to the stirring speed (N) and the diameter of the stirrer (D) as:

$\varepsilon = PV \alpha N^3 D^2$ (6)

Subsequently, the combination of the Eqs. (2) and (6) gives the following expression: dmean nanoparticle α dmean drop $\alpha \varepsilon^{-2/5}$ $(\sigma)^{3/5}$ (7)
(ρc)

Because Eq. (7) is valid for both scales, during the scaleup process it should be attempted to closely reproduce εpilot and εlab in order to obtain similar mean sizes of emulsion droplets at pilot- and lab-scales. In Fig. 6, the evolution of the mean diameter of the particles is plotted versus ε. As the batch volume increases, the mean size of NP shifts to smaller values. This drift indicates that the break-up phenomena is increased with the size of the batch, leading to smaller droplets in larger stirred tanks. In conclusion, scaleup induces smaller nanoparticles. This effect of scale-up on the droplet size of liquid–liquid turbulent dispersion has been already observed and widely discussed for emulsions in the micrometric range (Baldyga et al., 2001). However, in this study we have demonstrated that the same trend can also be encountered for emulsion systems in the nanometric size range.

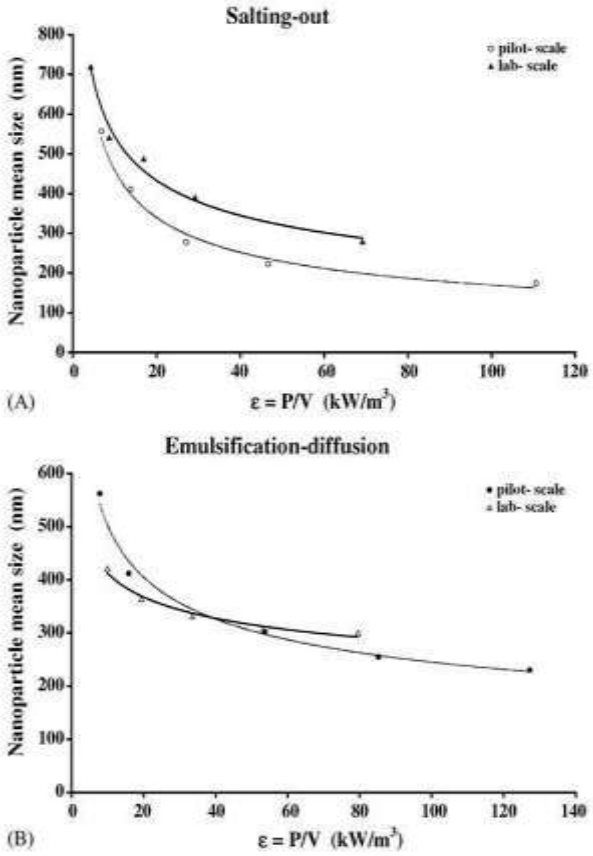

Fig. 6. Relationship between the specific power input of the stirrer and the nanoparticle mean size.

Laboratory and pilot production of nanoparticles by the nanoprecipitation method Nanoparticle formation during the nanoprecipitation method is governed by the "diffusion-stranding phenomena". In the early stage of this process, the solvent and polymer chains contained in the organic phase together diffuse into the continuous aqueous phase. Later, further diffusion of the solvent induces the desolvation of polymer chains which aggregate to form NP. As was previously shown (Galindo- Rodr´ıguez et al., 2004), any change in the solvent-diffusion behaviour leads to changes in the NP mean size. The characteristics of the aqueous and organic phases as well as the general procedure used

in this study can be seen in Table 1 and Fig. 1, respectively.

Nanoparticles produced at lab-scale manifested homogeneous populations characterized by their narrow size distributions. The NP mean size corresponded to 141±5 nm (mean±S.D., n = 3). In particular, the S.D. shows a low batch-to-batch variability. At pilot-scale, a continuous method (Fig. 3) was employed to prepare NP of reproducible sizes. The organic phase and aqueous phase were mixed in a ratio of 1:2 (v/v) by adjusting their flow rates to 62.5 and 125.0 ml/min, respectively. Under these conditions, average particle diameters of 105±8 nm and reasonably narrow size distributions were systematically obtained.

Table 5 Comparison of the nanoparticles prepared at the laboratory and pilot scales (mean ± S.D., n=3)

Nanoparticle characteristics [a]	Salting out		Emulsification–diffusion		Nanoprecipitation	
Mean diameter (nm)	274 ± 15	281 ± 5	293±15	287 ± 12	141 ± 5	105 ± 8
Polydispersity index[b] (0–1)	0.085 ± 0.012	0.091± 0.005	0.082 ± 0.021	0.116 ± 0.02	0.082 ± 0.023	0.130 ± 49
Repaglinide loading (%)	8.8 ± 0.3	7.5 ± 0.4	7.7 ± 0.2	5.5 ± 0.3	4.5 ± 0.3	3.2 ± 0.4
Entrapment efficiency[c] (%)	88	75	86	62	50	39
Residual PVAL (%)	5.6 ± 0.1	5.5 ± 0.4	4.8 ± 0.2	5.0 ± 0.1	3.7 ± 0.3	4.0 ± 0.4

[a] Three determinations were made for three different batches.

[b] For polydispersity index (PI), which ranges from 0 to 1, a higher value correspond to less homogeneous particle size distribution.

[c] Entrapment efficiency was the ratio of the experimentally measured Repaglinide content and the theoretical value expressed as a percentage.

A systematic difference (α 35 nm) in NP diameter was observed between the lab- and pilot-batches, which may be attributed to differences in the hydrodynamic conditions generated in the course of each manufacturing process. At lab-scale, NP are prepared by a batch system in which organic phase is poured into the aqueous phase under magnetic agitation. In contrast, at pilotscale, NP production is performed using a continuous system in which turbulence of the external phase is more pronounced. Since NP formation in the nanoprecipitation process depends on the diffusion of the solvent contained in the organic phase (Galindo-Rodr´ıguez et al., 2004), it is probable that the higher turbulence generated may improve the diffusion of solvent and hence the partition of the polymer chains into the aqueous phase, which would ultimately induce the formation of smaller NP. In general, the continuous method used for nanoprecipitation was the simplest scale-up process for producing NP. Moreover, pilot batches demonstrated good inter-batch reproducibility and required only 120 min to produce each batch (see Section 3.4). However, as already stated, the main drawback of this method was related to the low polymer concentration in the organic phase, which significantly limits the NP recovery in the final raw dispersion.

Influence of the scale-up process on the characteristics of nanoparticles: drug loading, residual PVAL and morphology.

In order to determine the effect of hydrodynamic conditions of the scale-up process on NP characteristics, NP of similar size were prepared at the lab- and pilot-scales and they were compared in terms of their drug loading, residual PVAL and morphology. For the emulsion-based methods, stirring rate was adjusted in order to obtain NP with a mean size of around 300 nm. In salting-out, although NP showed similar mean sizes (Table 5), lab- scale NP exhibited higher drug loading than those prepared by pilot-scale. These corresponded to 8.8 and 7.5%, respectively. A similar trend was observed in the emulsification–diffusion method: lab-scale and pilotscale batches had drug loadings of 7.7 and 5.5%, respectively. These findings suggest that the scale-up process slightly influence the drug loading of NP. In contrast, residual PVAL was found to be unaffected by the scale-up process (Table 5): the percentage of residual PVAL in batches prepared at lab- and pilot-scales was practically the same. This can be explained on the

basis of the mechanism of interaction of PVAL at the emulsion droplet interface and the NP mean size. It has been established that, during the emulsification and solvent diffusion steps of NP preparation, PVAL chains remain on the NP even after several washing cycles, essentially due to physical binding at the droplet interface between the PVAL and the polymer chains contained in the internal organic phase (Galindo-Rodr´ıguez et al., 2004). Since NP prepared by laband pilot-scales had similar mean size and specific surface area, the number of PVAL chains that can be adsorbed onto the NP will also be the same. As a result, residual PVAL values are similar. Finally, scanning electron micrographs of freeze-dried NP, prepared at the lab-scale and pilot-scale, are shown in Fig. 7. In the emulsification–diffusion method, although NP are not totally spherical (Fig. 7(A)), they appear to be homogeneous in size. The lack of roundness is probably due to the application of a vacuum during the freeze-drying stage. For salting-out, micrographs (Fig. 7(B)) showed spherical NP of sub-300 nm diameter with a relatively homogeneous size distribution. These results indicate that NP with similar morphological characteristics can be produced at both, lab- and pilot-scales.

In general, although this first approach can be considered acceptable, further studies should be conducted in order to optimize the scale-up processes of the emulsion-based methods. Considering differences in the drug loading, special attention should be paid to other influential variables such as the hydrodynamic conditions of the dilution step as well as the thermal similarity of the whole process. For the nanoprecipitation method, Repaglinide loading was significantly higher for the NP prepared at lab-scale (4.5%) than for those produced at pilot-scale (3.2%). This can be explained by differences in the "diffusion-stranding" phenomena of both systems. The higher turbulence in the continuous mode could have induced diffusion of the drug into the external aqueous phase before aggregation of the polymer chains to form the NP. Finally, the scanning electron microscopy analysis revealed the presence of spherical structures of around 100 nm for the pilot-scale and 100–140 nm for the lab-scale (Fig. 7(C)). In both cases, the particle size distribution was reasonably homogeneous.

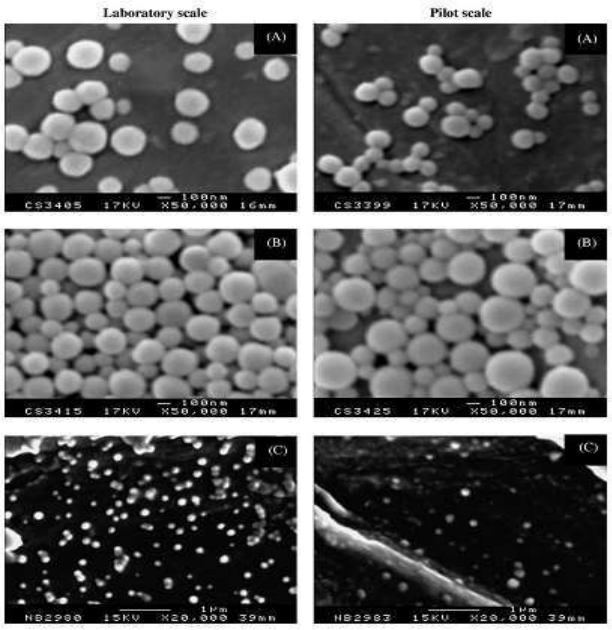

Fig. 7. Scanning electron micrographs of nanoparticles prepared, at laboratory and pilot scales, by (A) emulsification–diffusion (B) salting-out and (C) nanoprecipitation.

Time consumption for the three scale-up processes

Finally, the time taken by each technique to produce a single pilot-batch was also determined. The estimation was made by considering all operations involved from the preparation of raw solutions (aqueous and organic phases) to the formation of the raw NP dispersion (Fig. 8). It was noted that producing a pilot-batch by salting-out required less time (300 min) compared to emulsification–diffusion (350 min). Nevertheless, the shortest consumption time corresponded to the nanoprecipitation process, i.e. 120 min. Fig. 8 also illustrates that for the emulsion-based methods, the preparation of raw solutions is the critical step because it consumes more that 60% of the global production time. This is because high amounts of polymer, PVAL in the aqueous phase and Drug Coat L 100-55 in the organic phase, have to be dissolved in the raw solutions (Table 1). However, this is not completely unfavourable because a high polymer content in the organic phase enables a high recovery of NP in the final raw dispersion. Considering the composition of the organic phases for each method, 169.6, 100.0 and 18.0 mg of NP per ram of organic solvent can be potentially obtained by emulsification–diffusion, salting-out and nanoprecipitation, respectively.

Clearly, the two higher theoretical NP recoveries correspond to the emulsion-based methods.

Conclusions

This study shows that the scale-up of Repaglinide-loaded NP prepared by the salting-out, emulsification–diffusion and nanoprecipitation methods is feasible. First, by increasing the stirring rate, the mean size of nanoparticles can be systematically varied from 557 to 174 nm and from 562 to 230 nm for the pilot-batches prepared by salting-out and emulsification–diffusion, respectively. A well-known model used in the chemical engineering field was used for modeling the emulsification step of the two emulsion-based methods. This model, which is usually applied to liquid-liquid dispersions sized in the micrometer range and having a low volume fraction of dispersed phase, enables prediction of the NP mean size from the stirring rate. The accuracy of the model is better at pilot-scale (few liters). Moreover, the scale-up method is based on preserving similarities. When the classical criterion (namely the specific power input of stirring) used in emulsification is kept constant at both lab- and pilot-scales, the NP sizes are not constant. Larger scales of a few liters induce smaller NP sizes. With respect to the nanoprecipitation method, the continuous mode used for its scale-up allows production of NP in a facile and reproducible way. This method enables the preparation of batches of different volumes, from some milliliters to several liters, only by adjusting very few process parameters (e.g. flow rates of the organic and aqueous phase). Compared to the emulsion-based methods, less time is required for preparing a pilot-batch. Finally, it can be concluded that pilot-scale production of Repaglinide-loaded NP, prepared by salting-out, Emulsification–diffusion and nanoprecipitation, was relatively well achieved. In general, NP characteristics – drug loading, residual PVAL and morphology – were reproduced at lab- and pilot-scales. However, the scale-up process induced a slight reduction in the size and drug loading of NP.

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