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Influence of stabilizing agents and preparative variables on the formation of poly(D,L-lactic acid) nanoparticles by an emulsification-diffusion technique

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Abstract

Poly(D,L-lactic acid) (PLA) nanoparticles were prepared by a modified emulsification-diffusion technique. The preparation method consisted of emulsifying a solution of polymer in an aqueous solution of a stabilizing agent, previously saturated, followed by diluting the internal phase with an excess of water. Propylene carbonate (PC) was used as a partially water-miscible solvent, due to its ability to dissolve polymers, its low toxicity and its ease of emulsification. PC allowed the obtention of nanospheres in a reproducible and efficient way, using poly(vinyl alcohol) or poloxamer 188 as stabilizing agents. The possibility of using poloxamer 188 to produce PLA nanospheres is an interesting option because of its well known acceptability for parenteral administration. With other well accepted stabilizing agents such as polysorbate 80, gelatin, polyvinylpyrrolidone and dextran, it was not possible to obtain nanoparticles. The effectiveness of the stabilizing agents in the process was attributed to their ability to avoid coalescence during PC diffusion. The formation mechanism of nanoparticles can be explained by the large interfacial area resulting from emulsification and the gradual reduction of the globule size due to solvent transfer and probably, to the interfacial turbulence generated during diffusion. A higher concentration of polymer was found to rapidly increase the size and polydispersity of nanoparticles. In contrast, an increase in stirring rate and concentration of stabilizer agent were found to reduce moderately the size of the nanoparticles. Other process parameters such as viscosity, pH of the external phase and internal/external phase ratio had limited influence on particle size.

Keywords: Emulsification-diffusion method; Nanoparticles; Particle size; Poloxamer 188; Poly(D,L-lactic acid); Propylene carbonate

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1. Introduction

During the last few years, nanoparticles (nanospheres and nanocapsules) have become one of the most promising dosage forms for the controlled release of drugs. Particular attention has been paid to the development of parenteral therapeutic systems, made of biodegradable polymers, as potential formulations for site-specific drug delivery, including drug targeting. The study and technological development of these colloidal systems represent an important field in pharmaceutical research (Allémann et al., 1993a; Couvreur et al., 1995; Verrecchia et al., 1995).

In general, the methods for the preparation of nanoparticles can be classified into two main categories: polymerization of dispersed monomers and emulsification of natural macromolecules (such as albumin) or preformed polymers (pseudolatexes) (Couvreur et al., 1995). The presence of residual monomers, oligomers, surfactants and catalysts, as well as a possible cross-reaction with the drug, limit the use of the nanoparticles obtained by polymerization, primarily designed for parenteral administration (Gurny, 1983; Fessi et al., 1989; Couvreur et al., 1995). Well-established biodegradable polymers such as poly(D,L-lactic acid) or poly(D,L-lactic-*co*-glycolic acid) are commonly used in the preparation of nanoparticles, because of their biocompatibility and complete biodegradability (Aftabrouchad and Doelker, 1992; Schade et al., 1995; Couvreur et al., 1995).

Three techniques have been used for the preparation of nanoparticles based on preformed biodegradables polymers.

(1) The emulsion-evaporation procedure (Gurny et al., 1981), which consists in the emulsification of water-immiscible organic solutions of polymer in aqueous phases containing surfactants, followed by the removal of solvents under reduced pressure.

(2) The salting-out procedure (Bindschaedler et al., 1990; Allémann et al., 1992) based on the non-miscibility of a normally water-miscible solvent like acetone in saturated aqueous solutions, After preparing an oil/water emulsion (stabilized with poly(vinyl alcohol)), a certain quantity of water is added to allow the complete diffusion of acetone into the aqueous phase, thus inducing the formation of nanoparticles. (3) The nanoprecipitation procedure (Fessi et al., 1988), based on the addition of an acetone solution of polymer to a non-solvent solution.

Nevertheless, several difficulties have been encountered using these techniques when, for instance, working with toxic solvents (emulsification-evaporation), non i.v. accepted stabilizer agents i.e. poly(vinyl alcohol), and salts that are incompatible with bioactive compounds (salting-out). There can also be problems of low yields and poor entrapment efficacy (nanoprecipitation), (Allémann et al., 1993a; Verrecchia et al., 1995).

Recently, Leroux et al. (1995) developed a new method called emulsification-diffusion, using a partially water-soluble solvent, which is the well accepted benzyl alcohol. With this method, an oil/water emulsion is formed in the presence of stabilizing colloids, and the addition of sufficient quantity of water will induce the diffusion of the solvent and the precipitation of polymer as nanoparticles. The obtention of nanoparticles was possible using poly(vinyl alcohol) or gelatin as stabilizer. Drawbacks such as the non-biodegradability of poly(vinyl alcohol), as well as the high concentrations of stabilizer required, and the low yields obtained with gelatin, justify the study of the influence of other stabilizing agents on the process.

In this paper, the preparation and optimization of an emulsification-diffusion method for PLA nanoparticles, using propylene carbonate USP (PC) as a partially water-soluble solvent (0.25 g/ml) is investigated. PC has been reported to be a good solvent for polymers. Its oral and skin toxicity is low, it is chemically stable, practically odorless and forms emulsions of good physical and chemical stability (Meredith and Tobias, 1961; Stephens and Suddeth, 1967; Stephens and Felkel, 1975; Ong and Manoukian, 1988; Dahl and Burke, 1990).

The influence of some preparative variables on the nanoparticle size, such as the type and concentration of the stabilizing agent, the stirring rate, the internal/external phase ratio, the polymer concentration in the organic phase, the pH and the

viscosity of the external phase, have been investigated in order to control and optimize the process.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic acid) (PLA) (Medisorb®, 100 D,L, inherent viscosity 0.72 dl/g in chloroform), was supplied by Medisorb, (Cincinnati, OH, USA). Three nonionic stabilizing agents were tested: poly(vinyl alcohol) (PVAL) with molecular masses of 26 000 (Mowiol® 4-88, Hoechst, Frankfurt-am-Main, Germany); and PVAL 30 000–70 000 (Sigma, St. Quentin Fallavier, France); poloxamer 188 (Pluronic® F-68, BASF, Ludwigshafen, Germany) and polyoxyethylene sorbitan monooleate (polysorbate 80, Sigma). Other stabilizing colloids, dextran 70 (Fluka, Buchs, Switzerland), gelatin (Gelatina Alba Golddruck, Frankfurt-am-Main, Germany) and polyvinylpyrrolidone (Kollidon® 17, BASF) were tested at selected concentrations. Propylene carbonate (PC) of analytical grade was purchased from Fluka. Distilled water was of Milli-Q quality (Millipore, USA-Bedford, MD). All of the other reagents were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Nanoparticle preparation

PC and water (or buffer) were mutually saturated by each other for 1 min before use. Typically, 200 mg of PLA were dissolved in 10 ml of PC and this organic phase was emulsified with 20 ml of an aqueous surfactant solution (5% w/v) using a high speed homogenizer (Ultra-Turrax T25, IKA Labotechnik, Germany) at 8000 rpm for 10 min. Water (80 ml) was subsequently added under stirring to the emulsion in order to allow for diffusion of PC into the water, leading to the nanoprecipitation of the polymer. For some batches, the solvent was eliminated by cross-flow filtration, using a Minitan device (Millipore, USA-Bedford, MD) mounted with a polyvinylidene fluoride membrane with 0.1 μm pore size at a pressure of 0.9 bar, as previously described (Allémann et al., 1993b). The residual PC was monitored during the filtration process, according to a titration method (USP XXIII). After the collection of 4 l of diafiltered water, the quantity of PC became non-detectable but filtration was continued to 5 l, in order to make sure that the nanoparticle suspension was free of water-solubilized PC.

The batches produced with poloxamer 188 were filtered using a 0.1% w/v solution of the same surfactant, added in order to prevent flocculation. The PLA nanoparticles were finally frozen at -55°C for 10 min and freeze-dried (Lyolab BII, Secfroid, Aclens, Switzerland) under 0.05 mbar vacuum (condenser temperature < -60°C) for 24 h. The use of a cryoprotectant such as sucrose was necessary for batches produced with poloxamer 188. A schematic presentation of the nanoparticle preparation process is shown in Fig. 1.

2.2.2. Particle size analysis

For analysis of particle size 250 μl of nanoparticle suspension ($\sim$ 0.2% w/v) were diluted to 5 ml with water. The mean size and polydispersity (index from 0 to 9) were measured using a Coulter Nano-Sizer® (Coulters Electronics, Harpenden,

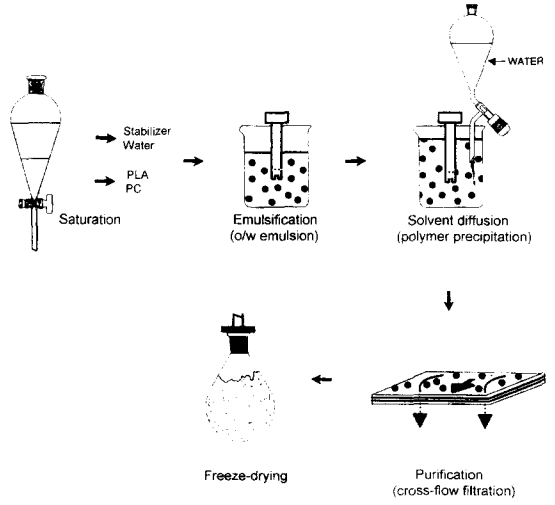

Fig. 1. Schematic representation of the nanoparticle preparation process.

Hertfordshire, UK). Measures were made in triplicate for all prepared batches.

2.2.3. Viscosity measurement

The viscosity of the PVAL solutions was determined using a rotating viscosimeter (Rheomat 15T-F Contraves, Zürich, Switzerland).

2.2.4. Scanning electron microscopy (SEM)

A concentrated aqueous dispersion of nanoparticles was finely spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with a gold layer (~ 20 nm thick). Then, the surface morphology of the nanoparticles was observed by SEM using a JSM-6400 scanning electron microscope (JEOL, Tokyo, Japan).

3. Results and discussion

3.2.1. Influence of the type of stabilizing agent

The proposed technique, seemingly simple, may involve a sequence of complex interfacial phenomena in which the type and the concentration of stabilizing agent play an important role in the formation of nanoparticles. A mechanism for the formation of PLA nanoparticles is suggested in Fig. 2.

After the mutual saturation of the two phases, the PC containing PLA and the water containing stabilizer, both liquids are in a state of thermodynamic equilibrium (a). Stirring causes the dispersion of the PC solution as globules in equilibrium with the continuous phase; the stabilizing agent is then adsorbed on the large interfacial area created (b). The formation of the o/w emulsion is necessary because PC does not spontaneously emulsify in water, as is the case with acetone in the nanoprecipitation process (Fessi et al., 1989). Therefore, superficial instabilities of interfaces can turn out to be only a necessary, but not a sufficient condition for spontaneous emulsification. The addition of water to the system (c) destabilizes the equilibrium. It causes the PC to diffuse to the external phase. During this transport of solute, new globules of nanometer size are produced

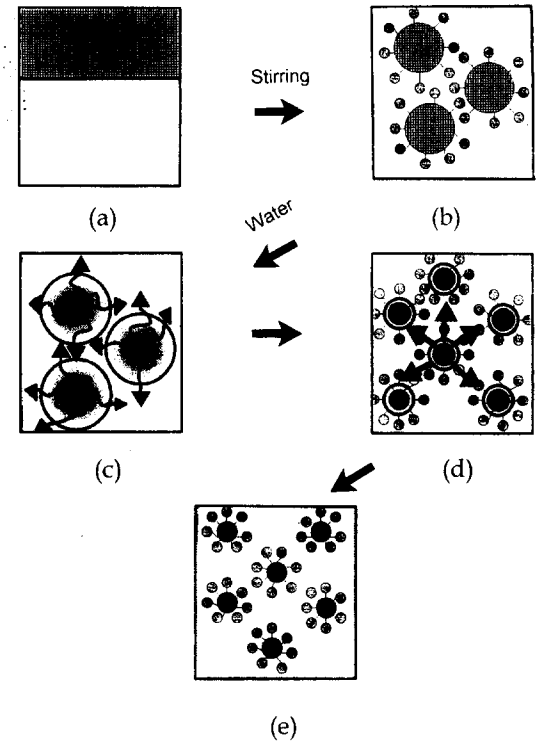

Fig. 2. Schematic description of the proposed formation mechanism of PLA nanoparticles by the emulsification-diffusion method (see text).

which gradually become poorer in PC (d). As a result, the polymer of the globules aggregates because of the presence of a new, continuous non-solvent phase. It is probable that the longitudinal variations (interfacial turbulence, Sterning and Scriven, 1959) generated during diffusion contributes, to a reduction of the globule size. Dimitrova et al. (1988) suggested that the stability of the drops, can be affected by the continuing solute mass transfer. The drops can collide and coalesce among themselves. Therefore, if the stabilizer remains at the liquid-liquid interface, during the diffusion process, and if its protective effect is adequate, then nanoparticles will form (e).

Fig. 3. shows the electron micrographs of the PLA nanoparticles produced with PVAL and poloxamer 188. The product consists of spherical and discrete particles in the nanometer size range.

The advantage of producing nanoparticles with poloxamer 188, lies in its low toxicity, even for parenteral administration (Schmolka, 1972; Koller and Buri, 1987). When polysorbate 80 was used as a surfactant, large aggregates were formed, independently of concentration and process conditions. Polysorbate 80 did not form stable PC/water emulsions, nor did it prevent globule coalescence during the diffusion of PC. Similar results were obtained in trials with gelatin, polyvinylpyrrolidone and dextran at concentration of 5% w/v. In preliminary tests with benzyl alcohol as solvent, it was not possible to obtain nanoparticles using poloxamer 188 at 5% w/v as stabilizer.

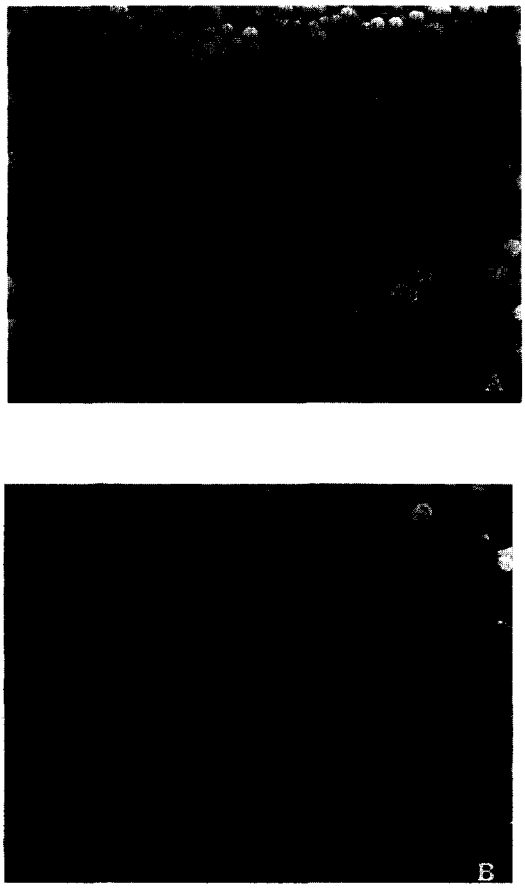

Fig. 3. Scanning electron micrographs of PLA nanoparticles. (a) PVAL 26 000, 5% w/v as stabilizing agent; (b) poloxamer 188, 5% w/v, as stabilizing agent.

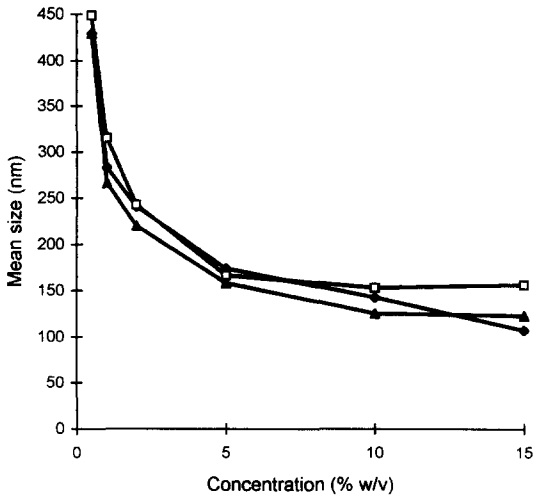

Fig. 4. Influence of the percentage of stabilizing agent in the external phase on the mean size of the nanoparticles. PVAL 30 000–70 000 (◆); PVAL 26 000 (□); poloxamer 188 (▲).

3.2.2. *Influence of stabilizer concentrations*

Stabilizer concentrations from 0.5 to 15% w/v of PVAL 26 000, PVAL 30 000–70 000 and poloxamer were used to evaluate the effect of emulsifier concentration on the nanoparticle size. The mean nanoparticle size was found to decrease sharply between 0.5 and 5% w/v of either stabilizer, but little change was observed above 5% (Fig. 4). Allémann et al. (1992) established that the final size of the nanoparticles in the process of salting-out depends on the globule size throughout the emulsification process. A reduction of the globule size allows the formation of smaller nanoparticles. The optimum packing concentration and the minimum globule size are already reached when the stabilizer concentrations are greater than 5% w/v. Above 5%, only a small quantity of stabilizer is adsorbed at the interface, the excess remains in the continuous phase, and does not play any significant role, neither in the emulsification nor in the protection of the droplets (Jalil and Nixon, 1990).

3.2.3. *Influence of viscosity and pH of the external phase*

Fig. 5 gives the relationship between the nanoparticle size and the viscosity of solutions of PVAL 26 000 and 30 000–70 000. Only at high

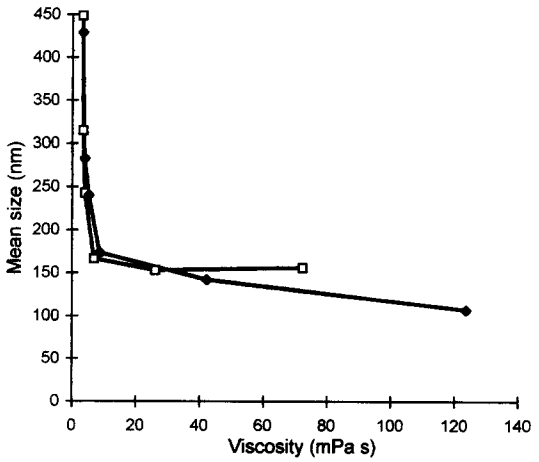

Fig. 5. Influence of the PVAL viscosity on the mean size of the nanoparticles (stirring rate 9000 rpm). PVAL 30 000–70 000 (◆); PVAL 26 000 (□).

concentrations of PVAL 30 000–70 000, there is an evident reduction of the nanoparticle mean size, from 174 nm (5% w/v; $\eta = 8.7$ mPa s) to 107 nm (15% w/v; $\eta = 123.7$ mPa s). This would suggest that the interfacial and mechanical properties (i.e. turbulent dispersion) play a more important role than does the viscosity of the external phase.

Nanoparticles were made using 0.1 M phosphate buffers with pH values ranging from 3 to 11 as dispersion and dilution solutions (Fig. 6). The modification of pH in the process could be potentially useful for improving the drug entrapment efficacy when preparing drug-loaded nanoparticles (Niwa et al., 1993; Allémann et al., 1993c).

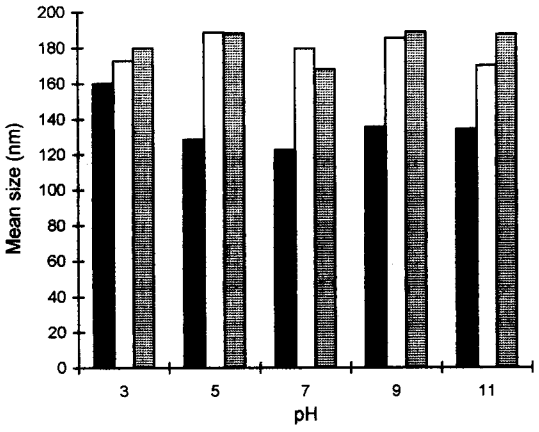

Fig. 6. Influence of the pH (dispersion and dilution solutions) on the mean size of the nanoparticles. Poloxamer 188 (■); PVAL 26 000 (□); PVAL 30 000–70 000 ( ).

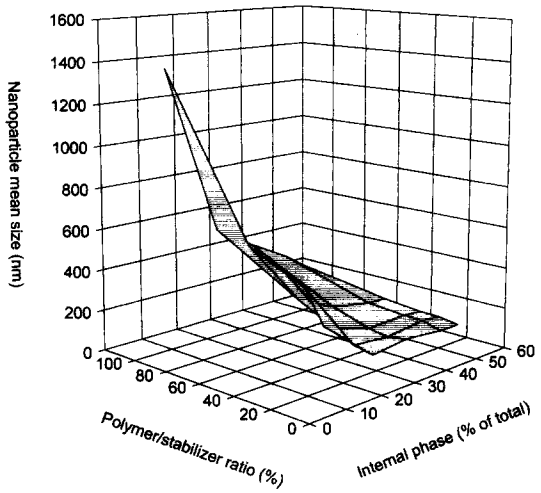

Fig. 7. Three-dimensional graph representing the effect of polymer/stabilizer ratio and internal phase on the mean size of the nanoparticles.

A reduction of the nanoparticle size was observed with poloxamer 188 for all the pH values. This phenomenon can be explained by the presence of electrolytes in solution. They produce a decrease in the hydration of poloxamer (Vadnere et al., 1984), thereby improving packing at the interface and reducing the globule size.

3.2.4. Influence of the PLA concentration and internal phase ratio

For these evaluations, poloxamer 188 was selected as stabilizing agent and used at a constant concentration of 5% w/v. PLA concentrations were varied from 10 to 50% w/v with respect to the amount of stabilizer in the aqueous phase. The internal phase (PC) varied from 9 to 50% v/v of the external phase. The experiments were planned using a simple latin square (25 experiments). The results are shown in Fig. 7 as a surface in a three-dimensional graph. It can be seen that an increase in the internal/external ratio leads to a slight decrease of the nanoparticle's average size for a particular polymer concentration. Coalescence of droplets can be prevented by a large quantity of PC. Apparently, a high quan-

tity of PC available for diffusion in the o/w emulsion prevents the coalescence of the droplets. The nanoparticles size and polydispersity increased when the polymer/surfactant ratio was higher. Polymer concentration in the internal phase was a crucial factor in increasing the size of nanoparticles, as its concentration was increased. The apparent mechanism responsible for this phenomenon is a high viscous resistance to the shear forces of emulsification. Coarse emulsions are obtained at high polymer concentrations, which lead to big particles during the diffusion process. Stolnik et al. (1994) have explained this fact by the greater probability that the desolvated macromolecules (or small aggregates formed from these molecules) coalesce, in a more concentrated solution, thereby forming larger coacervates or particles. Similar results were obtained by Leroux et al. (1995) using benzyl alcohol. In this case, the increase of the nanoparticle mean size was attributed to the difference in densities of the organic and aqueous phases.

3.2.5. *Influence of stirring rate*

Batches of nanoparticles using poloxamer 188 were prepared at 1000, 1480, 2000 and 2460 rpm, with a propeller stirrer (Heidolph-Elektro, KG type E-60, propeller: IKA 1381, Germany); and at 1500, 2000, 2460, 9000 and 13 500 rpm with the high speed homogenizer previously mentioned. As expected, a decrease of nanoparticle mean size and of polydispersity correlated with an increase of the stirring rate (Figs. 8 and 9). It was possible to obtain submicronic particles with both types of stirrers. At low stirring speeds with the high speed homogenizer, coarse emulsions were formed because of a poor stirring. The presence of aggregates and a wide size distribution were observed after the diffusion. Between 9500 and 13 500 rpm, a minimum size of ~ 150 nm was reached. A log-log plotting (boxes of Figs. 8 and 9) produced a straight line. Such graphs can be used to prepare nanoparticles of known average size by these preparation systems.

The impact of stirring in the processes of emulsion and diffusion was evaluated with the two stirrers at different stirring rates. The results drawn from this evaluation showed, on the one hand, that emulsification (discussed for the other parameters) is the most important process; and on the other hand, that a bad dispersion results in large nanoparticles with wide size distributions, irrespective of the stirring rate during diffusion (Table 1).

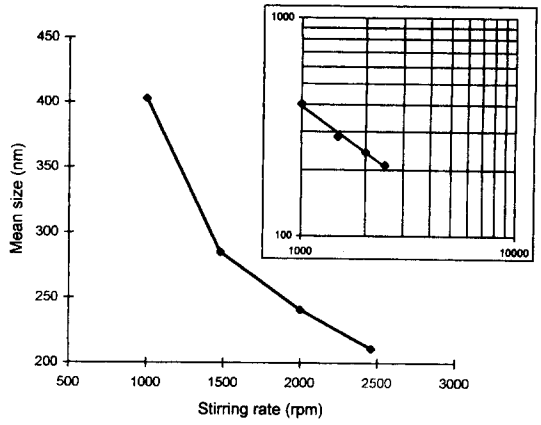

Fig. 8. Effect of stirring rate on the mean size of the nanoparticles prepared with a propeller stirrer and the corresponding log-log plot (box).

4. Conclusions

This study demonstrated how nanoparticles of well-characterized polymers can be produced by the emulsification-diffusion process, using par-

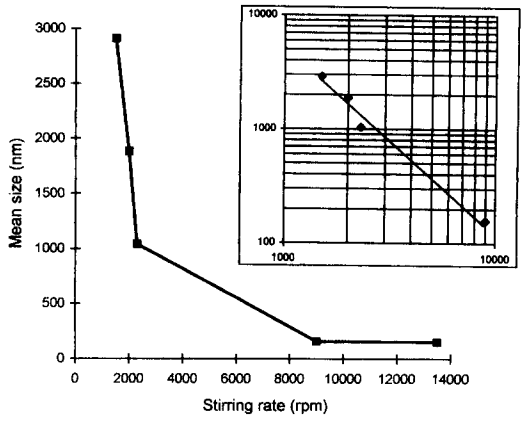

Fig. 9. Effect of stirring rate on the mean size of the nanoparticles prepared with a high speed homogenizer and the corresponding log-log plot (box).

Table 1
Influence of the stirring through the different process stages on the mean size of the nanoparticles

Emulsion		Diffusion		Mean size (nm) ($\pm$CV)[a]	Polydispersity[b]
Stirrer	Stirring rate (rpm)	Stirrer type	Stirring rate (rpm)		
Propeller	500	Homogenizer	9000	2180.3 (6.4)	9
Propeller	1000	Homogenizer	9000	591.2 (3.4)	6
Propeller	2000	Homogenizer	9000	231.8 (2.4)	1
Homogenizer	9000	Propeller	500	164.4 (0.4)	3
Homogenizer	9000	Propeller	1000	172.4 (0.4)	2
Homogenizer	9000	Propeller	2000	161.2 (2.2)	1

[a]CV: Coefficient of variation (%) ($n = 3$).
[b]index expressed from 0 to 9.

tially water-soluble solvents such as PC and non-ionic surfactants, acceptable for parenteral administration, like poloxamer 188. The emulsification-diffusion technique is an interesting alternative to existing methods because of its simple implementation, its reproducibility and the possibility of scaling up.

The nanoparticle formation process seems to be related to the interfacial area generated by emulsion formation and reduction of globule size due to the rapid diffusion of solvent. The corrugations or waves (interfacial turbulence) generated during diffusion, probably contribute to the globule size reduction and to the obtention of nanometric particles. The success of this method depends on the stability and on the size of the droplets, produced during the first stage of the process.

The nanoparticle size is influenced by the polymer concentration, stirring rate and stabilizer concentration. Other preparative variables such as viscosity, pH of the external phase and internal/external phase ratio had limited effect on particle size.

The study of the interaction of poloxamer 188 with the nanoparticle surface, the potential use of other partially water-soluble solvents and surfactants as well as the effect of drug's nature on the entrapment efficacy, are now under way.

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