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European Journal of Pharmaceutical Sciences 24 (2005) 67–75
EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES
www.elsevier.com/locate/ejps

Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles

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Received 17 May 2004; received in revised form 22 September 2004; accepted 27 September 2004
Available online 19 November 2004

Abstract

This study investigates formulation and process modifications to improve the versatility of the nanoprecipitation technique, particularly with respect to the encapsulation of hydrophilic drugs (e.g. proteins). More specifically, the principal objective was to explore the influence of such modifications on nanoparticle size. Selected parameters of the nanoprecipitation method, such as the solvent and the non-solvent nature, the solvent/non-solvent volume ratio and the polymer concentration, were varied so as to obtain polymeric nano-carriers. The feasibility of such a modified method was assessed and resulting unloaded nanoparticles were characterized with respect to their size and shape. It was shown that the mean particle size was closely dependent on the type of non-solvent selected. When alcohols were used, the final mean size increased in the sequence: methanol < ethanol < propanol. Surfactants added to the dispersing medium were usually unnecessary for final suspension stabilization. Changing the solvent/non-solvent volume ratio was also not a determinant factor for nanoparticle formation and their final characteristics, provided that the final mixture itself did not become a solvent for the polymer. A too high polymer concentration in the solvent, however, prevented nanoparticle formation. Both poly(lactic acid) (PLA) and poly(D,L-lactic-*co*-glycolic acid) (PLGA) could be used by accurately choosing the polymer solvent and in this respect, some non-toxic solvents with different dielectric constants were selected. The nanoparticles obtained ranged from about 85–560 nm in size. The nanoparticle recovery step however needs further improvements, since bridges between particles which cause flocculation could be observed. Finally, the presented results demonstrate that the nanoprecipitation technique is more versatile and flexible than previously thought and that a wide range of parameters can be modified.

Keywords: Entrapment efficiency; Nanoparticles; Nanoprecipitation; Protein; PLA; PLGA

1. Introduction

The nanoprecipitation technique (or solvent displacement method) for nanoparticle manufacture was first developed and patented by Fessi and co-workers (Fessi et al., 1989, 1992). This technique presents numerous advantages, in that it is a straightforward technique, rapid and easy to perform. The nanoparticle formation is instantaneous and the entire procedure is carried out in only one step. Briefly, it requires two solvents that are miscible. Ideally, both the polymer and the drug must dissolve in the first one (the solvent), but not in the second system (the non-solvent). Nanoprecipitation occurs by a rapid desolvation of the polymer when the polymer solution is added to the non-solvent. Indeed, as soon as the polymer-containing solvent has diffused into the dispersing medium, the polymer precipitates, involving immediate drug entrapment. The rapid nanoparticle formation is governed by the so-called Marangoni effect, which is due to interfacial turbulences that take place at the interface of the solvent and

Abbreviations: DMA, *N*,*N*-Dimethylacetamide; DMF, *N*,*N*-Dimethylformamide; DMSO, Dimethylsulfoxide; MeCN, Acetonitrile; MEK, Methyl ethyl ketone; MIBK, Methyl isobutyl ketone; NMP, *N*-Methylpyrrolidone

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doi:10.1016/j.ejps.2004.09.011

the non-solvent and result from complex and cumulated phenomena such as flow, diffusion and surface tension variations (Quintanar-Guerrero et al., 1998). Nanoprecipitation often enables the production of small nanoparticles (100–300 nm) with narrow unimodal distribution and a wide range of preformed polymers can be used, such as poly(D,L-lactic-*co*-glycolic acids), cellulose derivatives or poly ε-caprolactones. This method does not require extended shearing/stirring rates, sonication or very high temperatures, and is characterized by the absence of oily-aqueous interfaces, all conditions that might damage a protein structure. Moreover, surfactants are not always needed and unacceptable toxic organic solvents are generally excluded from this procedure.

However, the original nanoprecipitation method suffers from some drawbacks. This technique is mostly suitable for compounds having a hydrophobic nature such as indomethacin, which is soluble in ethanol or acetone, but displays very limited solubility in water. Consequently, reduced or even zero drug leakage toward the outer medium led to nanoparticles with entrapment efficiency values reaching 100% (Fessi et al., 1989; Barichello et al., 1999). However, recent research dealing with water-soluble drug incorporation has also provided encouraging results. Procaine hydrochloride, for instance, was more efficiently entrapped when the outer phase pH was set at a value that reduced drug ionisation and lowered its aqueous solubility (Govender et al., 1999), hence hampering considerably drug wastage by leakage. More recently, Yoo et al. (2001) have carried out experiments by slightly modifying the original concept, in order to encapsulate lysozyme. Briefly, they successfully effected the diffusion of a dimethylsulfoxide (DMSO) solution containing both the protein and the polymer (PLGA) into an aqueous solution of poloxamer 407. This work provided evidence that nanoprecipitation could also occur with solvents other than acetone or ethanol and thus that an accurate solvent and non-solvent selection (e.g. by screening) can also lead to nanoparticle formation and possibly extend the use of nanoprecipitation to more hydrophilic drugs.

In this respect, the present work essentially focuses on parameters of the nanoprecipitation procedure that might be modified in order to lead to the formation of nanoparticles and to extend the classical lipophilic drug/polymer/acetone or ethanol (solvent)/water (non-solvent) system to a more versatile drug/polymer/solvent/non-solvent scheme. For this purpose, we have mostly selected different alcohols as non-solvents, different chemical families as solvents (e.g. ketones and esters) and varied parameters such as the polymer concentration and the solvent/non-solvent ratio.

2. Materials and methods

2.1. Materials

Copolymers of poly(D,L-lactide-*co*-glycolide) (PLGA) with a 50/50 molar ratio with or without free carboxylic end groups (Resomer® RG 503, RG 503 H, M_w (GPC) of 34 kDa), as well as the poly(D,L-lactide) (PLA) homopolymer (Resomer® R 203, M_w (GPC) of 28 kDa) were purchased from Boehringer Ingelheim (Ingelheim am Rhein, Germany). A PLA with free carboxylic end groups (Medisorb PLA 100 DL 4A, M_w (GPC) of 57 kD) was obtained from Alkermes (Cincinnati, Ohio, USA). Poloxamer 407 (Lutrol® F127) was obtained from BASF (Ludwigshafen, Germany) and povidone K30 was purchased from Fluka Chemie (Buchs, Switzerland). Ethyl ether, methanol, *n*-propanol, isopropanol, *n*-butanol, isopropyl acetate, methyl acetate, methyl ethyl ketone and methyl isobutyl ketone were obtained from Aldrich Chemical (Buchs, Switzerland). Acetone, acetonitrile, ethanol, ethyl formate, *N*,*N*-dimethylformamide, *N*,*N*-dimethylacetamide, and *N*-methylpyrrolidone were purchased from Fluka Chemie (Buchs, Switzerland). 2-Pyrrolidone was a gift from BASF (Ludwigshafen, Germany). DMSO was obtained from Acros organics (Geel, Belgium). The purity of all these solvents was higher than 99%.

2.2. Nanoparticle preparation

The polymer was dissolved in a suitable organic solvent (S) at concentrations from 50 to 100 mg/mL to form the diffusing phase. This phase (with volumes typically ranging from 0.5 to 6 mL) was then added to the dispersing phase (5–20 mL) by means of a syringe positioned with the needle directly in the medium under moderate magnetic stirring. The dispersing phase was constituted from a liquid in which the polymer is insoluble – the non-solvent (NS) – optionally containing a surfactant (either poloxamer 407 or povidone K30). The freshly formed nanoparticles were then centrifuged four times for 15-min cycles at $15000 \times g$ and washed with distilled water, in order to gradually remove the dispersing medium and to replace it with water for subsequent scanning electron microscopy (SEM) characterization. The above procedure was used, unless otherwise stated.

2.3. Size determination

Particle size and polydispersity were determined by photon correlation spectroscopy (PCS) by using a Zetasizer 5000 (Malvern Instruments Ltd., UK). Each blank nanoparticle batch was appropriately diluted with the non-solvent just after production. Mean size and polydispersity were measured three times for each batch.

2.4. Nanoparticle morphology

The nanoparticle surface appearance and shape were analysed by SEM. Samples were prepared by finely spreading concentrated nanoparticle dispersions over slabs and by drying them under vacuum. The samples were then coated in

a cathodic evaporator with a fine gold layer and observed by SEM using a JSM-6400 scanning electron microscope (JEOL, Tokyo, Japan).

3. Results and discussion

3.1. Type of non-solvent

As shown in Tables 1 and 2, the final mean particle size was clearly dependent on the nature of the dispersing solvent. Indeed, methanol led to smaller nanoparticles than ethanol, as measured by PCS, whereas using *n*-propanol or isopropanol gave even larger nanoparticles (Fig. 1A and B). These size values were obtained with very high reproducibility (less than 4% of deviation between triplicates) and with polydispersity indexes that show very homogeneous nanoparticulate suspensions. When the solvent/non-solvent (S/NS) volume ratio and the polymer concentration were kept constant (i.e. 0.05 and 50 mg/mL, respectively), particle size gradually increased in the homologous alcohol series used as non-solvent. In this respect, it has been previously demonstrated that the rate of diffusion of the solvent into the non-solvent should certainly be considered, since the higher the rate of diffusion, the smaller the nanoparticles (and the higher the yield of transformed polymer into nanoparticles) (Stainmesse et al., 1992). Both *n*-butanol and ethyl ether did not enable nanoparticle formation. Indeed, as soon as the polymer solution was in contact with these non-solvents, the polymer formed a viscous gel and diffusion of the solvent into the non-solvent was impeded. In the case of ethyl ether, the polymer massively precipitated after a quick and pronounced desolvation. It should be mentioned that nanoprecipitation failure occurred mostly when the difference of the values of the dielectric constants between the solvent and the non-solvent was elevated. This reason has also been evoked by other authors, who found that the dielectric constant of the final solvent mixture was of importance (Thioune et al., 1997). A comparison between the two solvents MeCN and DMSO (Table 1 versus Table 2) shows that mean size values obtained with MeCN were always slightly larger than those obtained with DMSO. Moreover, diffusion into water was possible with DMSO, whereas nanoprecipi-

Table 1
Effect of the non-solvent on nanoparticle formation and mean size

Batch[a]	Non-solvent	Dielectric constant, ε	Surfactant (concentration)	Result[b]	Size ± S.D.[c] (nm)	PI[d] ± S.D.
1	Water	80.1 (20 °C)	–	–	–	–
2	Water	80.1 (20 °C)	Poloxamer 407 (1%)	–	–	–
3	Water:ethanol (50:50)	48.2 (37 °C)	–	–	–	–
4	Water:ethanol (50:50)	48.2 (37 °C)	Poloxamer 407 (1%)	–	–	–
5	Methanol	32.7 (25 °C)	–	+	203 ± 4	0.14 ± 0.03
6	Ethanol	24.6 (25 °C)	–	+	270 ± 2	0.11 ± 0.05
7	*n*-Propanol	20.3 (25 °C)	–	–	–	–
8	Isopropanol	19.9 (25 °C)	–	+	366 ± 10	0.06 ± 0.08
9	*n*-Butanol	17.5 (25 °C)	–	–	–	–
10	Ethyl ether	4.3 (20 °C)	–	–	–	–

[a] Solvent: MeCN, 1 mL. Polymer concentration: 50 mg/mL (Resomer RG 503). Non-solvent volume: 20 mL. S/NS volume ratio: 0.05.
[b] Key: (+) suspended nanoparticles, (−) complete polymer precipitation, (±) mixture of suspended nanoparticles and polymer precipitate.
[c] S.D.: standard deviation ($n = 3$).
[d] PI: mean polydispersity index expressed using a 0–1 scale ($n = 3$).

Table 2
Effect of the non-solvent, the S/NS volume ratio, and the surfactant on nanoparticle formation and mean size

Batch[a]	Non-solvent	Volume of non-solvent (mL)	S/NS volume ratio	Surfactant (concentration)	Result[b]	Size ± S.D.[c] (nm)	PI[d] ± S.D.
1	Water	20	0.05	–	+	174 ± 3	0.11 ± 0.01
2	Water	20	0.05	Poloxamer 407 (1%)	+	174 ± 0	0.06 ± 0.10
3	Methanol	20	0.05	–	+	102 ± 2	0.13 ± 0.03
4	Ethanol	20	0.05	–	+	224 ± 3	0.08 ± 0.02
5	Ethanol	5	0.2	–	+	227 ± 4	0.11 ± 0.02
6	Ethanol	5	0.2	Poloxamer 407 (1%)	+	228 ± 3	0.20 ± 0.01
7	*n*-Propanol	20	0.05	–	+	358 ± 2	0.05 ± 0.03
8	Isopropanol	20	0.05	–	+	377 ± 6	0.11 ± 0.07
9	Isopropanol	20	0.05	Povidone K30 (2%)	–	–	–
10	*n*-Butanol	20	0.05	–	–	–	–
11	*n*-Butanol	20	0.05	Povidone K30 (2%)	–	–	–

[a] Solvent: DMSO, 1 mL. Polymer concentration: 50 mg/mL (Resomer RG 503).
[b] Key: (+) suspended nanoparticles, (−) complete polymer precipitation, (±) mixture of suspended nanoparticles and polymer precipitate.
[c] S.D.: standard deviation ($n = 3$).
[d] PI: mean polydispersity index expressed using a 0–1 scale ($n = 3$).

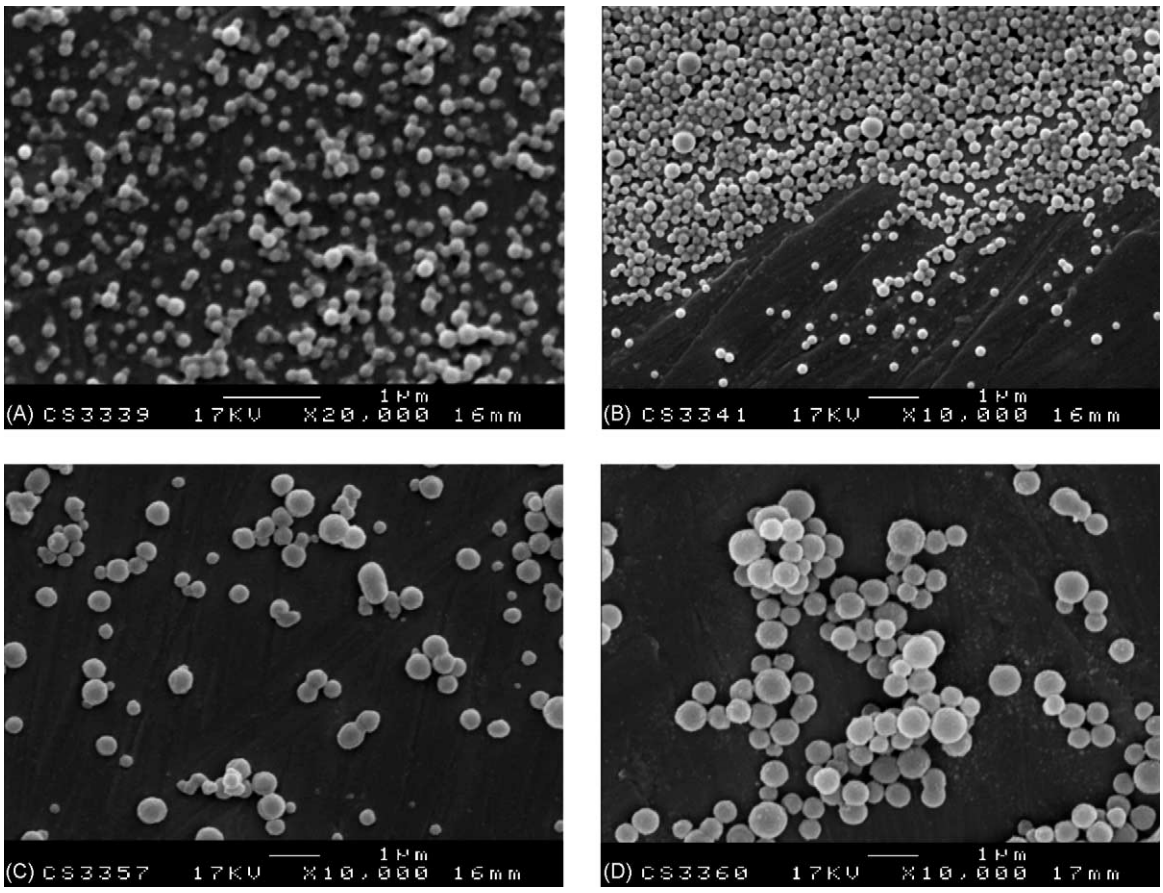

Fig. 1. Scanning electron micrographs of nanoparticles produced by nanoprecipitation under different conditions: (A) S = DMSO, NS = methanol, polymer = PLGA; (B) S = DMSO, NS = ethanol, polymer = PLA H; (C) S = methyl acetate, NS = ethanol, polymer = PLGA; (D) S = ethyl formate, NS = methanol, polymer = PLGA.

tation failed with MeCN. A hydro-alcoholic mixture (50:50) as non-solvent did not improve this result (batches 3 and 4, Table 1). Water-dispersed nanoparticles obtained in this way were also very small, with particle sizes close to those obtained with methanol and ethanol (batches 1–4, Table 2).

The addition of a surfactant usually had no influence on the outcome of nanoprecipitation (batches 1–4, Table 1), except on one occasion when a destabilizing effect was observed with povidone K30 on the final suspension leading to the formation of a white and compact polymer precipitate (batch 9, Table 2). Otherwise, no effect was noted on the final size as shown by batches 1–2 and 5–6, Table 2. Nanoparticles were also easily obtained when the S/NS ratio was brought to 0.2 instead of 0.05 (batches 5–6, Table 2).

The interest of using alcohols as non-solvents essentially lies in their relatively low dielectric constant (ε values). Indeed, the lower the dielectric constant value, the less the non-solvent will dissolve hydrophilic compounds, preventing drug leakage. Therefore, ethanol or *n*-propanol are the most suitable in this respect since their dielectric constant values are of 24.6 and 20.3, respectively, thus being far from that of the value for water (80.1). Moreover, using such liquids could be of interest for better protein molecular integrity protection. For example, the rate of spontaneous deamidation at asparagine residues was significantly reduced in solvents having low dielectric constant values (Brennan and Clarke, 1993). Finally, alcohols are not a concern in terms of toxicity, except for methanol. Indeed, they belong to Class 3 according to the ICH solvent toxicity scale (Class 3 solvents present very low risks to human health), whereas methanol appears amongst Class 2 solvents.

3.2. Polymer concentration and S/NS volume ratio

A too high polymer concentration in the solvent prevented nanoprecipitation (batch 1, Table 3). This effect is probably due to the high viscosity of the polymeric solution that hampers an appropriate diffusion of the solvent toward the non-solvent. This effect is overcome neither by the presence of a surfactant (batch 2), nor by augmenting the NS volume (batch 3). The formation of large aggregates due to high polymer concentration was also previously observed by other authors. Indeed, they found that the higher the polymer concentration in the solvent, the higher the loss of polymer. They

Table 3
Effect of the polymer concentration, the S/NS volume ratio, and the surfactant on nanoparticle formation and mean size

Batch[a]	Volume of solvent (mL)	Amount of polymer (mg)	Polymer concentration (mg/mL)	Volume of non-solvent (mL)	S/NS ratio	Surfactant	Result[b]	Size ± S.D.[c] (nm)	PI[d] ± S.D.
1	0.5	50	100	10	0.05	–	–	–	–
2	0.5	50	100	10	0.05	Poloxamer 407 (1%)	–	–	–
3	0.5	50	100	20	0.025	Poloxamer 407 (1%)	–	–	–
4	1	50	50	20	0.05	–	+	270 ± 2	0.11 ± 0.05
5	1	50	50	20	0.05	Poloxamer 407 (1%)	+	267 ± 6	0.21 ± 0.05
6	2	100	50	20	0.1	–	+	243 ± 4	0.08 ± 0.03
7	2	100	50	20	0.1	Poloxamer 407 (1%)	+	257 ± 6	0.07 ± 0.07
8	6	300	50	10	0.6	–	+	312 ± 6	0.06 ± 0.04
9	6	300	50	10	0.6	Poloxamer 407 (1%)	+	285 ± 2	0.02 ± 0.01

[a] Solvent: MeCN. Non-solvent: ethanol. Polymer: PLGA(Resomer RG 503).
[b] Key: (+) suspended nanoparticles, (−) complete polymer precipitation, (±) mixture of suspended nanoparticles and polymer precipitate.
[c] S.D.: standard deviation ($n = 3$).
[d] PI: mean polydispersity index expressed using a 0–1 scale ($n = 3$).

Table 4
Effect of the polymer on nanoparticle formation and mean size

Batch[a]	Polymer[b]	Non-solvent	Result[c]	Size ± S.D.[d] (nm)	PI[e] ± S.D.
1	PLGA H	Methanol	+	132 ± 1	0.13 ± 0.05
2	PLGA H	Ethanol	–	–	–
3	PLA	Methanol	+	133 ± 3	0.11 ± 0.05
4	PLA	Ethanol	+	318 ± 8	0.09 ± 0.03
5	PLA H	Methanol	+	111 ± 1	0.14 ± 0.02
6	PLA H	Ethanol	+	262 ± 3	0.03 ± 0.01

[a] Solvent: DMSO, 1 mL. Polymer concentration: 50 mg/mL (Resomer RG 503). Non-solvent volume: 20 mL (without surfactant). S/NS volume ratio: 0.05.
[b] Key: Resomer RG 503 (PLGA), Resomer RG 503 H (PLGA H), Resomer R 203 (PLA), Medisorb PLA 100 DL 4A (PLA H).
[c] Key: (+) suspended nanoparticles, (−) complete polymer precipitation, (±) mixture of suspended nanoparticles and polymer precipitate.
[d] S.D.: standard deviation ($n = 3$).
[e] PI: mean polydispersity index expressed using a 0–1 scale ($n = 3$).

explained this effect in terms of the intrinsic viscosity and interaction constants (Thioune et al., 1995, 1997; Stainmesse et al., 1995; de Labouret et al., 1995). In contrast, an increase in S/NS ratio by 12-fold (from 0.05 to 0.6) had no negative impact on nanoparticle formation (batches 4–9). This tends to demonstrate that this parameter should be preferentially modified (instead of polymer concentration) if a higher amount of nanoparticles is required in the final suspension. Moreover, it also enables a reduction of the total volume of solvent used. Again, the effect of poloxamer 407 on particle size was minor.

3.3. Type of polymer

The effect of the type of polymer on nanoprecipitation was also investigated. If a PLGA copolymer carrying more uncapped end groups was used, nanoprecipitation normally occurred in methanol, but not in ethanol where the polymer precipitated (batches 1 and 2, Table 4 versus batches 3 and 4, Table 2), contrasting with the PLA H performance (batch 6). The more hydrophobic PLA (with respect to PLGA) led to larger nanoparticles when diffusion was made possible into methanol (about 130 nm against 100 nm) and into ethanol (about 320 nm against 220 nm).

3.4. Type of solvent

As already observed for the non-solvent, the lower the dielectric constant of the solvent the larger the final nanoparticles (Tables 5 and 6). Again, the final particle sizes were always smaller with methanol than with ethanol and the smallest size was obtained with 2-pyrrolidone as solvent (batch 1, Table 5; 84 nm). It should be noted that nanoprecipitation with 2-pyrrolidone, NMP, DMF and DMA was possible into both methanol and ethanol and without surfactant (batches 1–8, Table 5). Actually, the dielectric constant of the solvent is certainly not only responsible for an increase of nanoparticle size, since nanoprecipitation results from various phenomena that govern the diffusion of the solvent through the polymer into the non-solvent. It is therefore expected that the choice of the solvent/non-solvent couple will affect the diffusion rate and thus the final mean size more than individual solvent characteristics like e.g., the dielectric constant, ε or the Hildebrand solubility parameter, δ. The affinity of the solvent for the non-solvent is of importance and, in this respect, the interaction parameter χ has certainly to be taken into consideration. This interaction is expressed as:

$$\chi = \frac{V_{NS}}{RT}(\delta_S - \delta_{NS})^2 \quad (1)$$

Table 5
Effect of the type of solvent and non-solvent on PLGA nanoparticle formation and mean size

Batch[a]	Solvent	Dielectric constant[b], ε	Solubility parameter[b], δ ($MPa^{0.5}$)	Non-solvent	Result[d]	Size ± S.D.[e] (nm)	PI[f] ± S.D.
1	2-Pyrrolidone	27.4[c]	30.1	Methanol	+	84 ± 1	0.14 ± 0.02
2	2-Pyrrolidone	NA	30.1	Ethanol	+	157 ± 3	0.14 ± 0.00
3	NMP	32.2	23.1	Methanol	+	116 ± 0	0.13 ± 0.01
4	NMP	32.2	23.1	Ethanol	+	260 ± 1	0.09 ± 0.04
5	DMF	36.7	24.8	Methanol	+	113 ± 2	0.15 ± 0.02
6	DMF	36.7	24.8	Ethanol	+	236 ± 5	0.13 ± 0.01
7	DMA	37.8	22.1	Methanol	+	115 ± 2	0.15 ± 0.01
8	DMA	37.8	22.1	Ethanol	+	268 ± 32	0.12 ± 0.08
9	Methyl acetate	6.7	19.6	Methanol	±	189 ± 5	0.08 ± 0.01
10	Methyl acetate	6.7	19.6	Ethanol	±	308 ± 13	0.07 ± 0.05
11	Ethyl formate	7.6	19.2	Methanol	+	299 ± 5	0.06 ± 0.05
12	Ethyl formate	7.6	19.2	Ethanol	±	525 ± 23	0.09 ± 0.05

[a] All batches were produced with 1 mL of solvent containing 50 mg of polymer (RG 503) and 20 mL of non-solvent without surfactant (S/NS ratio: 0.05).
[b] Values at 25 °C.
[c] Value at 31 °C.
[d] Key: (+) suspended nanoparticles, (−) complete polymer precipitation, (±) mixture of suspended nanoparticles and polymer precipitate.
[e] S.D.: standard deviation ($n = 3$).
[f] PI: mean polydispersity index expressed using a 0–1 scale ($n = 3$).

Table 6
Effect of the type of solvent and non-solvent on PLA nanoparticle formation and mean size

Batch[a]	Solvent	Dielectric constant, ε	Solubility parameter[c], δ ($MPa^{0.5}$)	Non-solvent	Result[d]	Size ± S.D.[e] (nm)	PI[f] ± S.D.
1	Acetone	20.7 (25 °C)	20.2	Methanol	+	168 ± 2	0.02 ± 0.03
2	Acetone	20.7 (25 °C)	20.2	Ethanol	+	385 ± 4	0.19 ± 0.15
3	MEK	18.5 (20 °C)	19.0	Methanol	+	164 ± 1	0.16 ± 0.03
4	MEK	18.5 (20 °C)	19.0	Ethanol	+	380 ± 11	0.20 ± 0.05
5	MIBK	13.1 (25 °C)	17.2	Methanol	+	243 ± 5	0.16 ± 0.09
6	MIBK	13.1 (25 °C)	17.2	Ethanol	+	558 ± 16	0.17 ± 0.06
7	Methyl propyl ketone	15.5 (20 °C)	17.8	Methanol	±	561 ± 40	0.82 ± 0.16
8	Methyl propyl ketone	15.5 (20 °C)	17.8	Ethanol	±	>1000	0.14 ± 0.04
9	Isopropyl acetate	NA[b]	17.2	Methanol	+	208 ± 4	0.25 ± 0.07
10	Isopropyl acetate	NA	17.2	Ethanol	+	443 ± 18	0.08 ± 0.06

[a] All batches were produced with 1 mL of solvent containing 50 mg of polymer (PLA, Resolmer R 203) and 20 mL of non-solvent free of surfactant (S/NS ratio: 0.05).
[b] NA = not available.
[c] Values at 25 °C.
[d] Key: (+) suspended nanoparticles, (−) complete polymer precipitation, (±) mixture of suspended nanoparticles and polymer precipitate.
[e] S.D.: standard deviation ($n = 3$).
[f] PI: mean polydispersity index expressed using a 0–1 scale ($n = 3$).

where V_{NS} is the molar volume of the non-solvent (here 40.7 cm^3/mol for methanol and 58.5 cm^3/mol for ethanol). The calculated interaction parameters (Table 7) were then plotted against nanoparticle size and presented in Fig. 2. As expected, the higher the interaction parameter, the larger the nanoparticles. It should be mentioned that the polymer was not taken into account here (and thus in the above formula), since both PLA and PLGA were previously shown to have very close δ values (Siemann, 1985). They are therefore expected not to affect final nanoparticle size in a significant fashion. This is also the reason why the results obtained with nanoparticles made from PLA can be plotted together with those obtained with nanoparticles made from PLGA (Fig. 2). Moreover, Choi et al. (2002) have already addressed the issue of the solvent–polymer interaction in a previous study. They demonstrated that the higher the interaction parameter $\chi_{solvent–polymer}$, the smaller the nanoparticles. It was claimed that a greater affinity between the solvent and the polymer led to more solvent remaining in the supersaturated polymer region. Therefore, this statement and the conclusion drawn from Fig. 2 confirm that the solvent motion toward the non-solvent is hampered by a greater affinity for the polymer and favoured by a greater affinity for the non-solvent. The solvents used to produce batches 1–8 (Table 5) all have high dielectric constant values (above 32) and are thus more prone to solubilize hydrophilic drugs than those with a lower ε value. They are all polar and aprotic solvents, as well as DMSO and MeCN. In this respect, such solvents are particularly interesting, as far as peptides or proteins are concerned (Chin et al., 1994). Indeed, good solubility is generally observed in DMSO (dielectric constant, ε at 25 °C: 46.6), whereas proteins are often poorly soluble in alcohols (e.g. insulin and lysozyme) (Stevenson, 2000). For the sake of comparison, the dielectric constant of MeCN (20 °C) is

Table 7
Calculated interaction parameters χ of solvent/non-solvent binary mixtures

Polymer	Non-solvent			
	Methanol		Ethanol	
	Binary mixtures (solvent/non-solvent)	Interaction parameter, χ[a]	Binary mixtures (solvent/non-solvent)	Interaction parameter, χ[a]
PLGA	MeCN	0.46	MeCN	0.11
	DMSO	0.43	DMSO	0.09
	2-Pyrrolidone	0.00	2-Pyrrolidone	0.31
	NMP	0.69	NMP	0.27
	DMF	0.38	DMF	0.07
	DMA	0.92	DMA	0.46
	Methyl acetate	1.64	Methyl acetate	1.12
	Ethyl formate	1.78	Ethyl formate	1.26
PLA	DMSO	0.43	DMSO	0.09
	Acetone	1.45	Acetone	0.94
	MEK	1.85	MEK	1.33
	MIBK	2.53	MIBK	2.04
	Methyl propyl ketone	2.29	Methyl propyl ketone	1.79
	Isopropyl acetate	2.53	Isopropyl acetate	2.04

[a] Calculated using Eq. (1) and for $T = 25\,^{\circ}C$ (298 K).

37.5. Moreover, it has been reported that proteins might be stabilized when dissolved in neat polar aprotic solvents such as DMSO. Most of the time, native conformation is then restored, because the conformational change is reversible upon reconstitution in water, as is often the case for enzymatic activity. In this respect, DMSO is even used to stabilize reversibly unfolded state (Singer, 1962). Hydrolytic degradation pathways are drastically limited in DMSO compared to water, as this is the case for leuprolide (Stevenson, 2000). Taking into account the above considerations, DMSO is thus the solvent of choice for obtaining protein-loaded nanoparticles by nanoprecipitation from both the protein solubility and stability points of view. In fact, protein entrapment with this method is still under investigation and this aspect will be addressed in a forthcoming paper. It should be noted, that the diffusing phase is not added drop-wise, but with the needle of the syringe directly in the non-solvent, in order to avoid an additional superfluous air–liquid interface that could adversely affect protein structure. Finally, DMSO is considered as non-toxic (in Class 3 of ICH classification) and 2-pyrrolidone is tolerated in parenteral formulation for veterinary use.

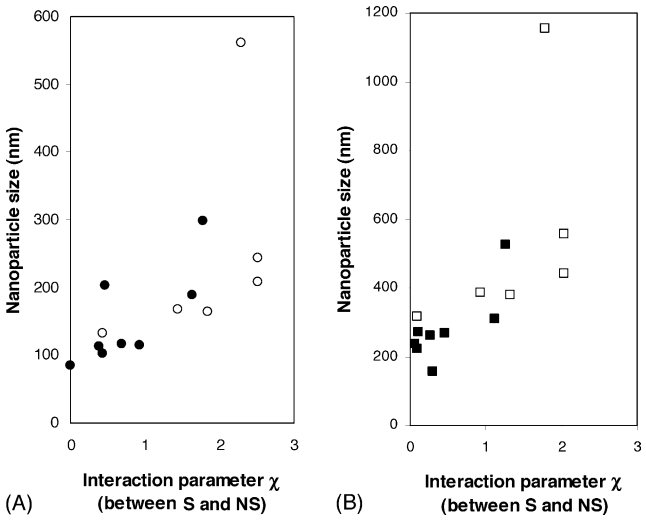

Fig. 2. Relationship between calculated interaction parameter χ of binary S/NS mixtures and nanoparticle size. (A) NS = methanol, (●) PLGA, (○) PLA; (B) NS = ethanol, (■) PLGA, (□) PLA.

The other solvents appearing in Table 5 (methyl acetate and ethyl formate) are able to dissolve the PLGA copolymer and have a far lower dielectric constant than the solvents cited above (Fig. 1C and D). They are therefore more adapted for lipophilic drug entrapment, but using them often leads to a mixture of very large nanoparticles (considered they were obtained by nanoprecipitation) and polymer precipitate.

Except for the ester isopropyl acetate, all the solvents listed in Table 6 are ketones. Among them, only acetone is commonly used for nanoprecipitation. They are all able to dissolve PLA homopolymer and have a lower dielectric constant value than methanol and ethanol, which destines them rather for lipophilic drug encapsulation. The highest particle size values were obtained with methyl propyl ketone (560 nm and more than 1 μm), but this solvent was also the only one, which did not lead to a fine and stable suspension. Similarly to the alcohols (used as non-solvents), methyl propyl ketone led to a less stable system (and also to larger particles) than methyl ethyl ketone. This tends to demonstrate that, in a family of homologous compounds, a higher number of carbons in the backbone formula is a destabilising factor.

3.5. Nanoparticle recovery

Before freeze-drying, nanoparticles normally have to be purified from residual products like surfactants, washed and resuspended in distilled water. If nanoparticles are large enough, centrifugation is used for the purification step. However, when the non-solvent was an organic solvent (e.g. an

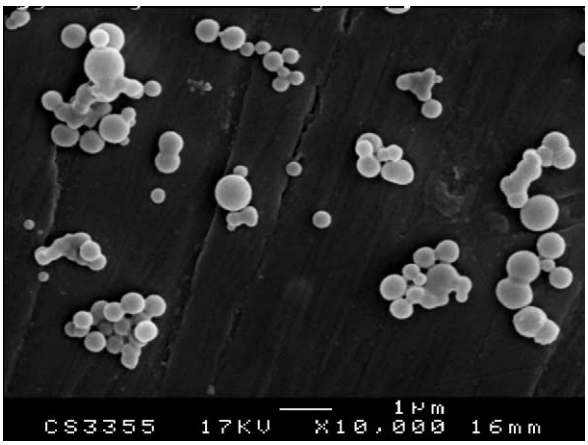

Fig. 3. Scanning electron micrograph of a nanoparticle batch produced by nanoprecipitation and centrifuged four times in order to replace methanol by water. S = ethyl formate, NS = methanol and polymer = PLGA.

alcohol), high speed centrifugation led to cakes which were not readily dispersible and centrifugation at a lower speed resulted in poor recovery yield (data not shown). Consequently, replacing the non-solvent by water was necessary. This step appeared to be a problematic hurdle, since nanoparticles might suffer from coalescence to some extent. As shown in Fig. 3, nanoparticles are bound together by interparticular bridges. As PCS measurements are relatively precise with respect to the values reported (and considering the standard deviations), it is assumed that this phenomenon occurred most of the time during the progressive replacement of the non-solvent by water, as described earlier. This observation is supported by a visible flocculation that takes place when water is added to the system. The fine nanoparticle suspension becomes turbid and can be easily centrifuged and resuspended, but this effect is obviously not desirable since it modifies nanoparticle morphology. As previously mentioned, the dielectric constant of the dispersing medium must certainly be taken into consideration and the incorporation of water to the system probably destabilizes the suspension.

4. Conclusions

The encouraging preliminary results presented in this study reflect the unexploited potential of the nanoprecipitation method for hydrophilic drugs or biopharmaceuticals. It has been demonstrated that a wide range of solvents for PLA/PLGA with different polarities can be used in order to enable the entrapment of hydrophilic or lipophilic drugs. Solvents like polar aprotic solvents, ketones or esters might be selected. DMSO was shown to be one of the most useful solvents, especially for protein drugs. It is a good non-toxic protein solvent and provides a stable environment to some extent. A suitable non-solvent can also be chosen on the basis of its polarity in order to favour final drug loading. In this respect, alcohols can provide nanoparticles with different sizes. It has been shown that small nanoparticles with narrow and regular distribution could be obtained. Moreover, surfactants are often not needed to stabilize the final nanoparticle suspension.

The nanoparticle recovery procedure, however, must still be optimized in order to prevent nanoparticle coalescence and to obtain an acceptable final nanoparticle yield. Aside from that, the results obtained by this modified nanoprecipitation method show great promise for protein encapsulation. Protein entrapment into nanoparticles with this nanoprecipitation method is currently under investigation and results will be presented in a forthcoming paper. Finally, it is assumed that the overall process could become a worthwhile option for nanoparticle production, even at an industrial scale.

Acknowledgement

This research was partially supported by a grant from the Roche Research Foundation, Basel.

References

Barichello, J.M., Morishita, M., Takayama, K., Nagai, T., 1999. Encapsulation of hydrophilic and lipophilic drugs in PLGA nanoparticles by the nanoprecipitation method. Drug Dev. Ind. Pharm. 25, 471–476.

Brennan, M., Clarke, S., 1993. Spontaneous degradation of polypeptides at aspartyl and asparaginyl residues: effects of the solvent dielectric. Protein Sci. 2, 331–338.

Chin, J.T., Wheeler, S.L., Klibanov, A.M., 1994. On protein solubility in organic solvents. Biotechnol. Bioeng. 44, 140–145.

Choi, S.W., Kwon, H.Y., Kim, W.S., Kim, J.H., 2002. Thermodynamic parameters on poly(D,L-lactide-*co*-glycolide) particle size in emulsification–diffusion process. Colloids Surf. A 201, 283–289.

Fessi, H., Puisieux, F., Devissaguet, J.-P., Ammoury, N., Benita, S., 1989. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int. J. Pharm. 55, R1–R4.

Fessi, H., Devissaguet, J.-P., Puisieux, F., Thies, C., 1992. Process for the preparation of dispersible colloidal systems of a substance in the form of nanoparticles. US Patent 593 522.

Govender, T., Stolnik, S., Garnett, M.C., Illum, L., Davis, S.S., 1999. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. J. Control. Release 57, 171–185.

de Labouret, A., Thioune, O., Fessi, H., Devissaguet, J.-P., Puisieux, F., 1995. Application of an original process for obtaining colloidal dispersions of some coating polymers: preparation, characterization, industrial scale-up. Drug Dev. Ind. Pharm. 21, 229–241.

Quintanar-Guerrero, D., Allémann, E., Fessi, H., Doelker, E., 1998. Preparation techniques and mecanisms of formation of biodegradable nanoparticles from preformed polymers. Drug Dev. Ind. Pharm. 24, 1113–1128.

Siemann, U., 1985. Proc. Int. Symp. Control Release Bioact. Mater. 12, 53.

Singer, S.J., 1962. The properties of proteins in nonaqueous solvents. Adv. Protein Chem. 17, 1–68.

Stainmesse, S., Orecchioni, A.-M., Nakache, E., 1992. Modelling of an original process to obtain biocompatible polymeric nanospheres. Proc. Sixth Congr. Int. Technol. Pharm. 1, 89–97.

Stainmesse, S., Orecchioni, A.-M., Nakache, E., Puisieux, F., Fessi, H., 1995. Formation and stabilization of a biodegradable polymeric colloidal suspension of nanoparticles. Colloid Polym. Sci. 273, 505–511.

Stevenson, C.L., 2000. Characterization of protein and peptide stability and solubility in non- aqueous solvents. Curr. Pharm. Biotechnol. 1, 165–182.

Thioune, O., Fessi, H., Wouessidjewe, D., Devissaguet, J.-P., Puisieux, F., 1995. Hydroxypropylmethyl cellulose phtalate aqueous dispersion for coating: preparation by a nanoprecipitation method. STP Pharma Sci. 5, 367–372.

Thioune, O., Fessi, H., Devissaguet, J.-P., Puisieux, F., 1997. Preparation of pseudolatex by nanoprecipitation: influence of the solvent nature on intrinsic viscosity and interaction constant. Int. J. Pharm. 146, 233–238.

Yoo, H.S., Choi, H.K., Park, T.G., 2001. Protein-fatty acid complex for enhanced loading and stability within biodegradable nanoparticles. J. Pharm. Sci. 90, 194–201.