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Preparation and characterization of poly-ε-caprolactone nanoparticles containing griseofulvin

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Abstract

Griseofulvin is an antifungal agent with poor solubility and low bioavailability. The aim of this work was to prepare poly-ε-caprolactone nanospheres and nanocapsules of griseofulvin by nanoprecipitation and to characterize them. Nanoparticles of griseofulvin were obtained with high encapsulation efficiency. The particle size was about 250–326 nm for nanospheres and 390–400 nm for nanocapsules.

The dissolution rate of griseofulvin nanoparticles was higher than that of micronized griseofulvin therefore recourse to nanoencapsulation of griseofulvin should enhance its bioavailability and possibly its efficiency for the treatment of dermatomycosis.

Keywords: Griseofulvin; Nanospheres; Nanocapsules; Preparation; Characterization; Dissolution rate

1. Introduction

Griseofulvin is an antifungal drug once used widely for the treatment of the dermatophytoses. Today it is used rarely because of the emergence of more effective antifungal drugs such as imidazoles and allylamines (Baran and Richert, 2003) and because the physicochemical properties of griseofulvin as a lipophilic molecule which is practically insoluble in water makes formulation and delivery difficult. However, this molecule still interests researchers. To enhance griseofulvin bioavailability, several trials are made in order to improve both its solubility and its dissolution rate. Indeed, it was proven that GF dissolution rate could be enhanced by micronization (Chaumeil, 1998), complexation of griseofulvin with cyclodextrin (Dhanaraju et al., 1998), preparation of griseofulvin nanoparticles from water-dilutable microemulsions (Trotta et al., 2003a) and preparation of griseofulvin nanosuspensions from triacetin-in-water emulsion (Trotta et al., 2003b). The use of bioadhesive polymer increases griseofulvin bioavailability (Khalid et

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al., 1997). Similarly griseofulvin solubility increases if solid solutions of griseofulvin are used with polyethylene glycol and sodium dodecyl sulphate (Wulff et al., 1995). It also increases when used in a preparation of vinylpyrrolidone/vinylacetate copolymer microspheres with griseofulvin (Vojnovic et al., 1993).

The main purpose of this piece of research was to prepare and characterize griseofulvin nanospheres and nanocapsules and to study the impact of nanoencapsulation on griseofulvin dissolution rate and solubility.

Griseofulvin nanoparticles were prepared by nanoprecipitation according to the method developed by Fessi et al. (1987, 1988) — by adding an acetone solution of polymer to a non-solvent solution. This method suits griseofulvin because of its lipophilic property and its solubility in acetone. Nanoparticles were characterized by determining their size, their external morphology and encapsulation efficiency.

2. Materials and methods

2.1. Raw materials

- griseofulvin in a micronized state (Sigma),
- polycaprolactone (PCL) with molecular weight of 80 000 (Aldrich),
- acetone (RP Normapur Prolabo),
- Tween® 80 (Seppic),
- Span® 80 (Seppic),
- benzyl benzoate (Merck),
- acetonitrile (HPLC Prolabo).

2.2. Preparation of nanoparticle suspensions

PCL, Span® 80, and griseofulvin were dissolved in acetone at 45 °C. This organic solution was injected at the rate of 48 ml/min in an aqueous phase containing water diluted Tween® 80 under magnetic stirring and at room temperature. Nanospheres were instantaneously formed by rapid solvent diffusion. Acetone and a large proportion of water were eliminated at 40 °C under reduced pressure to a final suspension volume of 30 ml. Nanocapsules were prepared using the same method with the addition of oil to the organic phase in which GF had been dissolved. It is worth noting that the oil to be used must be of good solvent property for the drug and must not degrade the polymer. Griseofulvin solubility was tested in different oils. Benzyl benzoate was found to be the best with a level of solubility of about 13.5 mg/ml.

Nanosphere suspension formulas were established (Table 1) with different griseofulvin concentration levels in order to obtain a higher encapsulation efficiency of nanosphere suspension. The nanocapsule formulas are shown in Table 2.

2.3. Nanoparticle characterization

2.3.1. Size analysis

The experiment was carried out on water dispersed micronized GF and PCL nanoparticle samples.

In order to analyze particle size, each suspension was diluted in distilled and filtered water solution with 20% of polysorbate 80. Particle size and polydispersity were determined by laser scattering light (Malvern S Instruments, UK), which allows sample measurement in the range 0.05–900 μm.

Polydispersity was determined according to the equation below:

$$\text{Polydispersity} = \frac{D(0.9) - D(0.1)}{D(0.5)}$$

where $D(0.9)$ corresponds to particle size immediately above 90% of the sample. $D(0.5)$ corresponds to particle size immediately above 50% of the sample. $D(0.1)$ corresponds to particle size immediately above 10% of the sample.

Nanoparticle size and polydispersity were determined both after stirring particle suspensions and after centrifugation at 3000 rpm during 15 min to eliminate excess polymer and free griseofulvin. Each sample was measured in triplicate.

2.3.2. External morphological study

External morphology of nanoparticles was determined using transmission electron microscopy (TEM) Topcon® EM 002B, 200 kV. Usually the samples are prepared by placing one preparation drop on a collodion support on copper grids (Malaiya and Vyas, 1988), followed by negative staining with an aqueous sodium phosphotungstate solution (Al-Khouri Fallouh et al., 1986; Rollot et al., 1986; Seijo et al., 1990), phos-

Table 1
Formulas of nanosphere suspensions

Samples	Griseofulvin (mg)	PCL (mg)	Span® 80 (mg)	Acetone (ml)	Tween® 80 (mg)	Water (ml)
NS_0	0	138	50	25	100	50
NS_1	1.38	138	50	25	100	50
NS_2	2.76	138	50	25	100	50
NS_3	4.14	138	50	25	100	50
NS_4	5.52	138	50	25	100	50
NS_5	6.90	138	50	25	100	50
NS_6	8.28	138	50	25	100	50
NS_7	9.66	138	50	25	100	50
NS_8	11.04	138	50	25	100	50
NS_9	12.42	138	50	25	100	50
NS_{10}	13.80	138	50	25	100	50

photungstic acid (Fessi et al., 1989) or uranyl acetate.

2.4. Encapsulation efficiency of nanoparticles

Total griseofulvin was determined after full dissolution of a specific amount (1 ml) of griseofulvin-loaded PCL nanoparticle suspension in 20 ml of acetonitrile and filtration with a 100 nm filter syringe.

Free griseofulvin was determined in the sediment after centrifugation of the samples at 9000 rpm during 30 min (Sigma K 16). The sediment was then dissolved in acetonitrile and free griseofulvin dose was determined after filtration with a 100 nm filter syringe.

All free griseofulvin precipitates after centrifugation of the samples at 9000 rpm during 30 min due to the larger size of micronized griseofulvin and also its very poor water solubility. However, nanoparticles remain floating.

GF encapsulation efficiency was calculated as indicated below:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{total amount of griseofulvin} - \text{free griseofulvin}}{\text{total amount of griseofulvin}} \times 100$$

2.5. Storage stability

Nanoparticle suspensions were stored under static conditions at 4 and 25 °C during a period of 6 months. Stability was assessed by comparing the initial encapsulation efficiency and particle size with those obtained after 6-month storage at 4 and 25 °C. The results of experiments were checked for statistical significance using the statistical analysis (Student's *t*-test) where the differences are considered insignificant when $p > 0.05$.

2.6. Griseofulvin solubility

A study of solubility was made for micronized griseofulvin as well as griseofulvin-loaded PCL nanospheres and nanocapsules to investigate the effect of nanoencapsulation on the solubility of griseofulvin. Based on the results of the nanosphere encapsulation efficiency, we retained the sample with the largest quantity of encapsulated griseofulvin.

Three suspensions were used. The first one contains excess of micronized griseofulvin in distilled water, the second one a concentrated suspension of nanospheres in water and the third one is a concentrated suspension of nanocapsules in water. The suspensions were stirred at 25 °C during 48 h and filtered with a 100 nm membrane (Millipore) under reduced pressure. The solu-

Table 2
Formulas of nanocapsule suspensions

Samples	Griseo-fulvin (mg)	PCL (mg)	Benzyl benzoate (ml)	Span® 80 (mg)	Acetone (ml)	Tween® 80 (mg)	Water (ml)
NC_0	0	138	0.5	100	25	100	50
NC_1	6.5	138	0.5	100	25	100	50

tions were then assayed for griseofulvin concentration by HPLC after acetonitrile extraction. Each griseofulvin batch was analyzed in triplicate.

2.7. Dissolution study

This study is carried out on three suspensions: the first one contains nanospheres with the largest quantity of encapsulated griseofulvin; the second one contains griseofulvin nanocapsules and the third one contains micronized griseofulvin. The suspensions were diluted in distilled water to a final volume of 1000 ml so that griseofulvin concentrations were about 6 μg/ml in compliance with the sink conditions. The samples were incubated at 25 °C under gentle magnetic stirring at 500 rpm. We removed 10 ml of the medium at regular intervals and replaced them by 10 ml of fresh water. The aliquots were filtered with a 100 nm membrane (Millipore) under reduced pressure. Concentration of dissolved griseofulvin was determined by HPLC after extraction with acetonitrile. The assays were carried out in triplicate.

2.8. HPLC determination of griseofulvin

The quantitative determination of griseofulvin in acetonitrile was performed by high performance liquid chromatography HPLC (Hewlett Packard). A UV detector was used for spectrophotometric analysis. Separation was achieved by using a reversed-phase column (25 cm × 4.6 mm; C18 Spherisorb ODS 2) with a flow rate of 1.5 ml/min. The mobile phase consisted of a mixture of acetonitrile and water in the ratio of 65:35. The UV detector was set at 291 nm. A standard curve was constructed for griseofulvin in the concentration range of 0.5–50 μg/ml. A linear relationship was observed between the concentration of griseofulvin and the peak area ($r^2 = 0.9996$). The retention time was found to be 2.4 min. An amount of 20 μl of each sample was injected after filtration with a 0.1 μm filter syringe.

3. Results and discussion

3.1. Particle size analysis

Nanoparticle suspensions revealed a unimodal size distribution. Two typical size distribution curves are shown in Fig. 1. After centrifugation the mean size of particles was in the range 250–326 nm for nanospheres and 390–400 nm for nanocapsules (Table 3 refers).

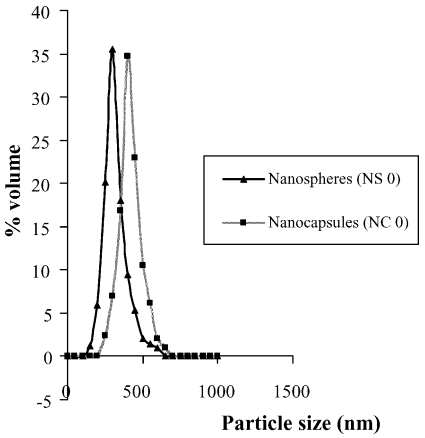

Fig. 1. Typical size distribution of PCL nanospheres and PCL nanocapsules.

According to available research and literature, three factors are deemed essential in the ultimate determination of nanoparticle size; namely, the concentration of polymer in the organic phase (Fessi et al., 1987), the polarity of the solvents (Thioune et al., 1997) and the internal/external phase ratio (Wehrle et al., 1995).

3.2. External morphological study

The external morphological study revealed that all nanoparticles were spherical and all of them were amorphous (Fig. 2).

In Fig. 2a the arrows show the nanocapsules on the carbon layer. Their apparent diameter was higher than their real diameter, most probably because of their flattened shape.

In Fig. 2b the arrows A and B show the nanospheres captured in the hole of carbon. Arrow C shows an empty hole of carbon without any nanosphere.

3.3. Encapsulation efficiency of the nanoparticles

The percentages of nanosphere encapsulation efficiency are inversely proportional to griseofulvin concentration (Table 4 refers). However, sample NS_9 contains the largest quantity of encapsulated griseofulvin. Therefore, NS_9 was used as a reference to study griseofulvin dissolution rate and solubility.

Table 3
Particle size and polydispersity of micronized griseofulvin and of nanoparticles

Samples	Size after stirring of the suspension (nm), size ± S.D.[a]	Polydispersity after stirring of the suspension, PI ± S.D.[a]	Size after centrifugation (nm), size ± S.D.[a]	Polydispersity after centrifugation, PI ± S.D.[a]
Micronized griseofulvin	4330 ± 121	1.970 ± 0.24	–	–
NS_0	406 ± 11	2.676 ± 0.712	266 ± 11	0.4632 ± 0.049
NS_1	356 ± 11	1.165 ± 0.062	296 ± 5	0.3483 ± 0.063
NS_2	416 ± 15	1.941 ± 0.604	293 ± 15	0.4507 ± 0.075
NS_3	403 ± 5	3.256 ± 0.020	310 ± 10	0.3284 ± 0.063
NS_4	390 ± 10	1.745 ± 0.529	250 ± 17	0.3056 ± 0.032
NS_5	463 ± 5	1.858 ± 0.359	306 ± 5	0.4299 ± 0.035
NS_6	383 ± 5	3.123 ± 0.135	313 ± 5	0.3358 ± 0.029
NS_7	403 ± 5	1.104 ± 0.793	286 ± 15	0.3361 ± 0.014
NS_8	426 ± 5	1.481 ± 0.499	293 ± 5	0.3373 ± 0.026
NS_9	393 ± 5	2.794 ± 0.332	303 ± 15	0.4007 ± 0.017
NS_{10}	410 ± 10	3.992 ± 0.698	326 ± 5	0.3891 ± 0.011
NC_0	506 ± 11	1.083 ± 0.140	400 ± 10	0.2527 ± 0.012
NC_1	523 ± 5	1.542 ± 0.286	390 ± 12	0.2635 ± 0.045

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

The percentage of nanocapsule encapsulation efficiency is more important than that of nanospheres with the same quantity of drug; but in the case of nanocapsules the quantity of griseofulvin is limited by its solubility in the benzyl benzoate.

3.4. Storage stability

Nanoparticle suspensions maintained the initial properties with respect to size and encapsulation efficiency after six months of storage at 4 and 25 °C, respectively ($p > 0.05$).

3.5. Griseofulvin solubility

According to Table 5, the solubility of griseofulvin after encapsulation decreased despite particle size reduction of griseofulvin and the presence of Tween® 80 and Span® 80 in the nanoparticle suspensions. This can be explained by the fact that bulk of griseoful-

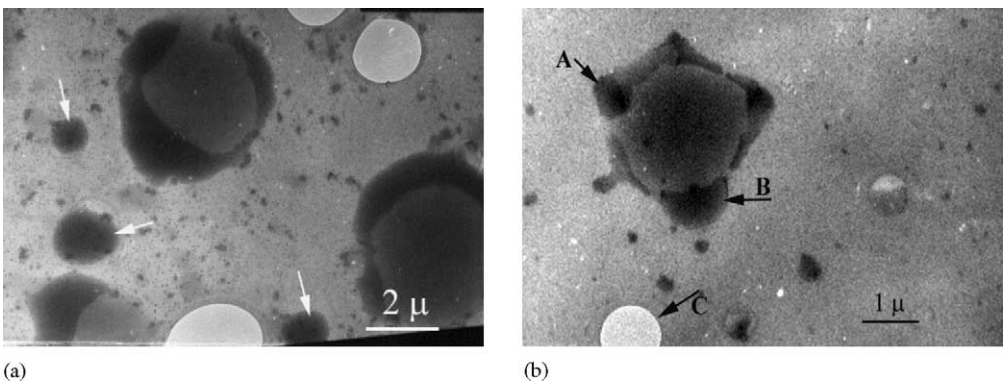

Fig. 2. Transmission electron microscopy of: (a) nanocapsule suspension and (b) nanosphere suspension.

Table 4
Encapsulation efficiency of nanoparticles

Samples	Encapsulation efficiency (%), % ± S.D.[a]	Quantity of encapsulated griseofulvin (mg)
NS_0	–	–
NS_1	98.48 ± 1.65	1.359
NS_2	98.59 ± 1.03	2.721
NS_3	97.89 ± 1.77	4.052
NS_4	91.01 ± 1.32	5.023
NS_5	89.02 ± 0.89	6.160
NS_6	85.93 ± 2.09	7.115
NS_7	82.21 ± 1.49	7.941
NS_8	80.60 ± 1.23	8.898
NS_9	78.20 ± 0.65	9.712
NS_{10}	68.90 ± 1.02	9.508
NC_0	–	–
NC_1	98.36 ± 1.92	6.431

[a] $n = 6$; SD: standard deviation between the six assays.

vin was adsorbed or trapped inside the nanoparticles and smaller quantity of the drug remained free in the medium and moved to a molecular state.

3.6. Dissolution study

According to Table 6, the dissolution rate of the griseofulvin was enhanced by recourse to encapsulation compared to that of micronized griseofulvin.

Table 5
Griseofulvin solubility in water at 25 °C (μg/ml)

	Solubility (μg/ml)
Micronized griseofulvin	30.56 ± 0.26
Nanocapsules of griseofulvin	11.48 ± 0.51
Nanospheres of griseofulvin	12.71 ± 0.32

Table 6
Percentages of dissolved griseofulvin from micronized griseofulvin, griseofulvin nanospheres and nanocapsules

Time (min)	Percentages of dissolved griseofulvin (%)		
	Micronized griseofulvin	Griseofulvin nanospheres	Griseofulvin nanocapsules
5	13.85 ± 0.54	87.24 ± 2.12	98.50 ± 0.52
10	19.63 ± 1.00	99.17 ± 0.90	100.91 ± 1.27
15	25.86 ± 0.72	101.36 ± 1.09	100.42 ± 0.65
30	30.92 ± 1.80	100.94 ± 1.66	101.07 ± 1.27
45	37.87 ± 1.89	101.16 ± 1.27	100.67 ± 0.56
60	43.18 ± 2.52	100.32 ± 0.75	101.64 ± 0.61
90	56.70 ± 2.83	100.79 ± 0.63	101.15 ± 0.59
120	61.23 ± 1.64	100.66 ± 1.93	100.62 ± 0.51

In sink conditions the release of griseofulvin from nanospheres and nanocapsules was very rapid and complete. In fact 100% of the drug was released after 15 min. For nanocapsules, the release is only governed by the partition coefficient of the drug between the oily core and the aqueous external medium and the relative volumes of both phases. The rate of diffusion of the drug through the thin polymeric barrier does not seem to be a limiting factor (Legrand et al., 1999). For nanospheres the release depends, on the one hand, on the mechanism of encapsulation (the release is faster if the drug was adsorbed by the nanospheres than if it was entrapped inside the matrix) and on the other hand on the rate of polymer biodegradability.

The presence of Span® 80 and Tween® 80, respectively in the organic phase and in the aqueous phase should intervene in the rate of release of the drug from encapsulated drug. Indeed, Xiaohong et al. (1999) have demonstrated that the rate of release of Leptospira Interrogans antigens from poly-DL-lactide-poly (ethylene glycol) microspheres can be enhanced when the concentrations of Tween® 80 and Span® 80 in the medium are increased. Nanoparticle size influences the dissolution rate which increases as particle size decreases, due to an increase of the available surface area of the drug particles (Trotta et al., 2003b).

4. Conclusion

This study confirms that nanoprecipitation is suitable for the preparation of griseofulvin nanoparticles, in view of the fact that high encapsulation efficiency was obtained.

For nanosphere suspensions, the quantity of encapsulated griseofulvin depends on its concentration vis-à-vis the polymer. In this case, the optimal percentage of griseofulvin compared with polymer was found to be 9%.

For nanocapsule suspensions, however, the quantity of encapsulated drug depends on the solubility of griseofulvin in benzyl benzoate.

Griseofulvin nanoparticles showed a higher dissolution rate of the drug which suggests that lower doses of this molecule could be used for oral applications reducing thus its side effects.

On the basis of the finds of this research and considering that percutaneous absorption was improved

when the drugs were nanoencapsulated — as demonstrated by some authors (Jiménez et al., 2004; Richart and Simmonet, 2003), we can conclude that there is a large scope for improving the use of griseofulvin for dermatophytose treatments either through oral administration or by percutaneous application.

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