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Optimized preparation of poly D,L (lactic-glycolic) microspheres and nanoparticles for oral administration

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Abstract

A rotatable central composite design (RCCD) was applied to optimize the preparation of cyclosporine-loaded poly D,L (lactide-glycolide) (PLAGA) nanoparticles (NP) and microspheres (MS) by solvent displacement and solvent evaporation techniques, respectively. The joint influence of needle gauge, polymer amount and the injection rates on the mean particle size, relative standard deviation (RSD), yield and drug encapsulation percentage in NP were evaluated. With regards to MS, the polymer amount and the stirring rate were evaluated. Scan electron microscopy of MS and NP showed spherical particles with a dense polymeric network in the first case. From the statistical analysis of data polynomial equations were generated. The mean particle size ranged from 50 to 150 nm for NP and from 1.5 to 30 μm for MS. Smallest nanoparticles (46 nm) were obtained by using the lowest polymer amounts, the highest injection rates and the lowest needle gauges ($r^2 = 0.9443$). Under these conditions the drug entrapment percentage was maximum (85.2%), suggesting the drug might be entrapped and adsorbed on the nanoparticle surface. The relative standard deviation was only affected by the polymer amount ($r^2 = 0.8034$) and the yield rose with the amount of PLAGA ($r^2 = 0.9016$). A very important increase in particle size ($r^2 = 0.9855$), relative standard deviation ($r^2 = 0.9353$) and encapsulation percentage ($r^2 = 0.9669$) were observed for MS by decreasing emulsification stirring rates and increasing polymer amounts, the stirring rate being the most significant independent variable ($\alpha < 0.0001$) in all cases. The highest experimental encapsulation value ($97.69 \pm 0.78\%$) correspond to samples prepared from 150 mg of polymer and a global stirring rate of 2000 rpm. By using response surface diagrams and the mathematical models proposed, it is possible to easily deduce experimental conditions to prepare NP and MS with the desired properties.

Keywords: Nanoparticles; Microspheres; Experimental Designs; Cyclosporin A; Poly D,L (lactide-glycolide)

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1. Introduction

Cyclosporin A (CyA) is a cyclic undecapeptide with a potent immunosuppressive activity which has been used to prevent graft rejection in organ transplant recipients (Ptachcinski et al., 1986). It is usually administered by the oral and intravenous route. Its major drawbacks are the dose-related nephrotoxicity and the low and variable oral bioavailability. To improve drug efficiency, it has been incorporated into liposomes (Gruber et al., 1989; Vadiei et al., 1989; Stuhne-Sekalec and Stanacev, 1991; Akbarieh et al., 1993; Al-Angary et al., 1995), microemulsions (Mueller et al., 1994), enteric solid dispersions (Takada et al., 1989) or lipid microspheres (Yanagawa et al., 1989). CyA is also used to treat a widespread number of autoimmune diseases at different levels, such as uveitis or Chron's disease. So alternative routes for drug administration are now under thorough study to increase drug efficacy in these pathologies (Sandborn et al., 1991; Keenan et al., 1992; Oh et al., 1995).

The advantages of nanoparticles (NP) in the ocular administration of drugs are well known (Harmia et al., 1987; Losa et al., 1993; Marchal-Heussler et al., 1993) as is the potential ability of microspheres (MS) to target drugs to the colon (Jani et al., 1994) or to improve the percutaneous absorption of drugs by the transfollicular pathway (Rolland et al., 1993). NP and MS have also been suggested to reduce the nephrotoxicity of CyA by means of controlling drug release and modifying drug distribution after subcutaneous or intravenous administration (Sánchez and Alonso, 1995; Bonduelle et al., 1995). Polymeric NP also increase the CyA oral bioavailability (Molpeceres, 1994) while drug nephrotoxicity remained invariant despite the higher CyA blood levels (unpublished observation). Orally administered NP and MS are taken up by the lymphoid associated tissue (Peyer's patches) in the GI tract (Kreuter, 1991), and upon intravenous administration they are captured by the rethyculo-endothelial system (passive vectorization) (Bonduelle et al., 1995) which is a partial target for an immunosuppressive drug. Nevertheless, they are also susceptible of specific targeting to T-lymphocytes decreasing the drug dose needed to achieve immunosuppression (Rolland et al., 1987).

In spite of the numerous potential applications of NP and MS, their sizes must be tailored in accordance with the requirements of each administration route and to their therapeutic aim. Jani et al. (1990) reported an inverse relationship between nanoparticle size and oral bioavailability, and the studies by Eldridge et al. (1990) showed microspheres larger than 10 μm in diameter were not retained by the Peyer's patches of the small intestine. Rolland et al. (1993) also suggested a size related skin distribution of the MS. Moreover, an optimal drug to polymer ratio needs to be achieved in order to diminish the toxicity of these systems (Illum et al., 1986). In this sense, poly D,L-lactic and glycolic acid copolymers (PLAGA) have been extensively used because of the total absence of toxicity of their degradation products and their modulatable degradation rates (Vert et al., 1994).

Much attention has focused on MS preparation while NP preparation methods have been scarcely studied. Recently, we reported on the joint effects of some technological and formulation parameters on the characteristics of CyA-loaded polycaprolactone (PCL) NP prepared by solvent displacement (Molpeceres et al., 1996). Other NP preparation methods, such as the polymerization of isobutylcyanoacrylate (Alonso et al., 1990) or glutaraldehyde (McLeod et al., 1988), the solvent evaporation (Julienne et al., 1992) or a modified solvent displacement method (Wehrle et al., 1995) have been systematically studied by using experimental designs.

In summary, in order to prepare PLAGA NP and MS to be lately administered in vivo, a previous rotatable central composite design (RCCD) has been used to evaluate the joint influence of different processing variables on NP and MS characteristics. The aim of these studies was to develop a mathematical model in order to deduce the adequate conditions to prepare colloidal systems of desired characteristics, which could improve the oral bioavailability of cyclosporine and reduce its associated nephrotoxicity.

2. Materials and methods

2.1. Materials

Cyclosporin A was supplied by courtesy of Sandoz (Basle, Switzerland); Poly D,L (lactide-glycolide) 50:50 was purchased from Boehringer Ingelheim S.A. (Germany). Polyvinyl alcohol (PVA) was obtained from Sigma (St. Louis, MO) and Pluronic^R F-68 (Poloxamer 188) from Fluka Chemika (Switzerland). Methylene dichloride (Panreac, Spain) and acetone (Scharlau, Spain) and all other reagents were of analytical grade.

2.2. Preparation of NP and MS

CyA loaded PLAGA NP were prepared by the method of Fessi et al. (1989) which was slightly modified by using a glass bladder-device designed in our laboratory (Molpeceres et al., 1996), in order to control the organic phase injection rates, with drug and polymer, into the aqueous phase. Thus, the injection rate will be the result of combining different needle diameters and varying gas pressures, which were constantly monitored. Temperature (20°C), surfactant amount (100 mg), acetone and water volumes (20 and 40 ml), final drug concentration (100 μg/ml) and stirring rate (500 rpm) were kept constant.

MS were prepared by the solvent evaporation method (Bodmeier and McGinity, 1987; Jeffery et al., 1991), keeping constant the time taken to form the emulsion (5 min), the volume ratio for the organic to aqueous phase (4.5/12.5) and the final drug (100 μg/ml) and surfactant concentration (PVA 0.6%). In both cases the organic solvent was eliminated under reduced pressure and the final volume of the aqueous suspension was adjusted to 10 ml.

2.3. Particle size analysis

The mean particle size and the standard deviation (SD) for NP were measured by using a Microtrac^R ultrafine particle analyzer (Leeds and Northrup, Ireland) (range from 5 nm to 2.75 μm) which works on the principle of dynamic light scattering. MS diameter were determined in a Microtrac^R standard range analyzer (Leeds and Northrup, Ireland) based on the principle of laser diffraction analysis with a particle size range from 0.69 to 704 μm.

2.4. Morphological characterization

Scanning electron microscopy (SEM) (Zeiss DSM 950) was used to visualize NP and to evaluate the size, shape and surface characteristics of MS. MS internal structure was also studied by inclusion into Spurr resins. Transmission electron microscopy (TEM) (Zeiss EM 10) was employed to characterize NP by using a negative staining.

2.5. Encapsulation efficiency

Drug concentrations were analyzed by a reverse phase HPLC method, previously described (Guzmán et al., 1993). The CyA entrapped in the colloidal carrier was calculated by the difference between the total amount of drug in the samples and that in the external aqueous phase after ultracentrifugation of the suspensions at 40 000 *g* and 4°C during 1 h for NP and 11 000 *g* during 30 min for MS. The encapsulation efficiency was expressed as the percentage of drug incorporated in the carrier relative to the total amount of drug in the medium. The yield was referred to as the ratio between the experimentally measured amount of drug in the suspension and the theoretical quantity of drug used.

2.6. Experimental design

A rotatable central composite design (RCCD) was applied in order to optimize the preparation of NP and MS. The needle gauge (mm) (X_1), the global injection rate (ml/s) (X_2) and the amount of polymer (mg) (X_3) have been evaluated for NP. MS's factors were the polymer amount (mg) (Y_1) and the stirring rate used for emulsification (rpm) (Y_2). In both cases a five levels RCCD was used. Tables 1 and 2 show the correspondence between the orthogonal and the real values for the variables involved in NP and MS preparation, respectively. They have been distributed at random in experimental blocks, which should be carried out

Table 1
Independent variables and their correspondence between real and orthogonal values in the central composite design for NP preparation

	Real values		
Levels	Needle gauge X_1 (mm)	Injection rate X_2 (mL/s)	Polymer amount X_3 (mg)
−1.68179	0.5	0.11	20
−1	0.7	0.207	52.4
0	0.8	0.35	100
1	0.9	0.492	147.56
1.68179	1.1	0.59	180

in different days so as to estimate the experimental variability. All the preparations, 20 for NP and 14 for MS, were obtained in triplicate ($n = 3$), and the dependent variables were determined twice in each one. The reproducibility in the experimental work was determined from the samples prepared in the central conditions (a total of 6 samples in 3 batches) and the resulting coefficients of variation were less than 7.5%.

2.7. Statistical analysis

A mathematical relationship between factors and parameters has been generated by polynomial regression analysis (NCSS) (Hintze, 1992). To check the validity of the regression model (González, 1993) some tests were applied. Statistically significant F-ratios ($\alpha < 0.05$) and correlation coefficients higher than 0.9 or correlation coefficients between 0.8 and 0.89 associated to non statistically significant lack of fits ($\alpha > 0.05$) are the criteria for validation of the models chosen, according to those previously suggested by Wehrle et al. (1995), and slightly modified in order to achieve a higher statistical significance for validation. The canonical analysis gives the stationary point (González, 1993) and finally, a significance test for the regression coefficients was performed so as to obtain the regression equations including only the terms with statistical significance. They were also represented as three-dimensional response surface plots for two variables at a time.

Table 2
Independent variables and their correspondence between real and orthogonal values in the central composite design for MS preparation

Levels	Real values	
	Polymer amount Y_1 (mg)	Stirring rate Y_2 (rpm)
−1.41421	100	2 000
−1	114.64	4 400
0	150	10 000
1	185.35	15 600
1.41421	200	18 000

3. Results and discussion

The RCCD constitute an alternative approach since it offers the possibility of investigating a higher number of variables at different levels, performing only a limited number of experiments. The variables selected were chosen taking into account those in other studies dealing with nanoparticles or microparticles preparation methods (Bodmeier and McGinity, 1987; Fessi et al., 1989; Jeffery et al., 1991; Julienne et al., 1992; Molpeceres et al., 1996; Wehrle et al., 1995).

Table 3 shows the average experimental results concerning the joint tested variables on particle size, relative standard deviation (RSD), drug encapsulation and yield for NP, RSD being the sample standard deviation expressed as percentage of the mean particle size. The mean particle size ranged from 50 to 146 nm. Thus, a very fine control of NP size can be achieved by combining the three independent variables. The CyA entrapment percentage and the yield ranged from 47.90 to 84.71% and from 53.82 to 92.06%, respectively. The statistical analysis of the results generated the following polynomial equations, that were validated according to the criteria presented in Section 2:

Table 3
Orthogonal and response values of different variables obtained from the analysis of poly D,L (lactide-glycolide) nanoparticles. Results are the mean of three assays determined twice

Formulation	Orthogonal values			Response values			
	X_1	X_2	X_3	Mean size ± SD (nm)	Relative standard deviation (%)	Encapsulation rate (%) ± SD	Yield (%) ± SD
1	−1	−1	−1	73 ± 12	26	67.73 ± 6.66	58.25 ± 11.06
2	−1	1	1	83 ± 23	35	84.71 ± 2.67	82.07 ± 9.71
3	1	−1	1	146 ± 21	34	79.69 ± 3.32	88.16 ± 6.00
4	1	1	−1	72 ± 17	27	69.00 ± 2.39	72.07 ± 2.92
5	0	0	0	102 ± 20	34	78.32 ± 2.72	82.28 ± 3.50
6	0	0	0	97 ± 12	35	78.77 ± 2.85	76.56 ± 6.78
7	−1	−1	1	121 ± 35	38	82.84 ± 3.77	92.06 ± 7.16
8	−1	1	−1	50 ± 14	27	71.24 ± 1.03	73.55 ± 6.11
9	1	−1	−1	81 ± 1	30	67.81 ± 2.36	61.43 ± 19.15
10	1	1	1	119 ± 28	36	82.69 ± 2.79	79.19 ± 2.96
11	0	0	0	97 ± 10	38	78.61 ± 1.24	77.17 ± 2.47
12	0	0	0	99 ± 29	31	76.23 ± 3.87	73.05 ± 3.92
13	1.682	0	0	117 ± 16	34	72.66 ± 3.89	87.49 ± 7.91
14	−1.682	0	0	46 ± 9	33	83.68 ± 2.73	80.98 ± 2.05
15	0	1.682	0	62 ± 4	33	80.54 ± 0.65	83.13 ± 8.06
16	0	−1.682	0	123 ± 6	32	77.31 ± 2.31	73.47 ± 19.26
17	0	0	1.682	120 ± 15	37	83.97 ± 1.14	77.90 ± 8.30
18	0	0	−1.682	67 ± 21	22	47.90 ± 7.64	53.82 ± 18.25
19	0	0	0	96 ± 20	30	74.65 ± 1.41	69.07 ± 7.15
20	0	0	0	99 ± 22	30	74.49 ± 4.90	81.19 ± 1.97

X_1, needle gauge (mm); X_2, injection rate (ml/s); X_3, polymer amount (mg).

$$\text{Mean size (nm)} = 98.1457 + 15.4298X_1 - 14.5973X_2 + 20.6186X_3 - 4.9202X_1^2 + 3.2062X_2X_1 + 3.8395X_3X_1 - 3.8979X_3X_2$$

$$r^2 = 0.944306 \quad (1)$$

$$\text{RSD (\%)} = 33.0549 + 4.2651X_3$$

$$r^2 = 0.803429 \quad (2)$$

$$\text{\% Encapsulation} = 76.7968 - 1.8944X_1 + 8.4058X_3 - 3.5362X_3^2$$

$$r^2 = 0.926908 \quad (3)$$

$$\text{\% Yield} = 76.5583 + 8.5422X_3 - 3.8347X_3^2 - 5.6121X_3X_2$$

$$r^2 = 0.901649 \quad (4)$$

where X_i represent the orthogonalized values of the independent variables: needle gauge (X_1), global injection rate (X_2) and PLAGA amount (X_3).

The three-dimensional response surface plots for the most statistical significant variables on the mean size of NP are shown in Fig. 1a,b. Fig. 1c shows the effect of needle gauge and polymer amount on drug encapsulation. By using higher polymer amounts, slower injection rates and larger needle gauges, particles association takes place and the largest particles with a broad RSD were obtained. The amount of polymer had a more important impact on mean particle size than on RSD as is shown by the respective F associated values ($F = 20.30$, $\alpha < 0.001$ and 7.26, $\alpha = 0.0286$).

A theoretical model (Molpeceres et al., 1996) has been previously developed to rationalize polycaprolactone NP formation by solvent displace-

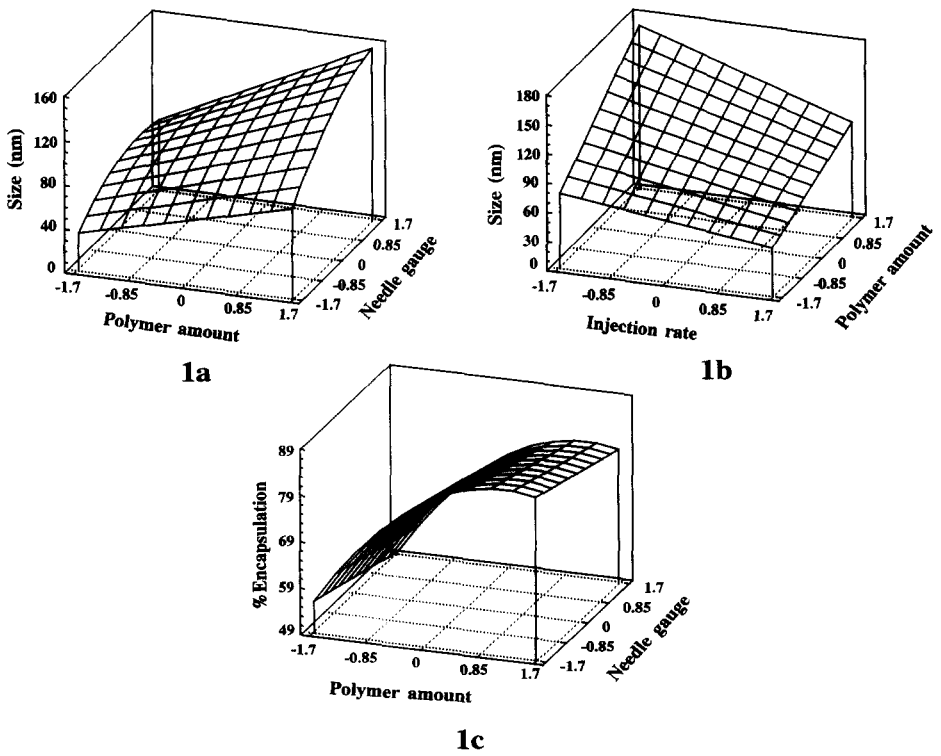

Fig. 1. Three-dimensional response surface plots showing the variation in the NP size (nm) (a) (b) and the drug encapsulation percentage (c) with changes in needle gauge (X_1), injection rate (X_2) and polymer amount (X_3).

ment in order to predict the mean particle size from the formulation conditions:

$$\text{Size} = K \cdot [\text{Ct} \cdot S/2 \cdot \text{GRi}]^{1/2} \quad (5)$$

where Ct is the concentration of PCL in the acetonic phase, S is the cross-section of the needle used in the injection and the global rate of injection GRi is the volume of organic solvent (ml) that goes into the water per second. This model were tested for PLAGA NP and a similar qualitative results were obtained, founding a statistical significative linear relationship (slope = 345.22, $r^2 = 0.839$) (Fig. 2). The small differences in the K value obtained for polycaprolactone nanoparticles (Molpeceres et al., 1996) (slope = 520.01, $r^2 = 0.891$) could be attributed by polymer characteristics.

With regards to the encapsulation, percentage of CyA entrapped in PLAGA nanoparticles depended linearly to the needle gauge and quadratically to the polymer amount ($\alpha < 0.0001$, Fig. 1c). The highest drug entrapment percent was obtained with the highest quantity of PLAGA and the smaller needle gauge. These conditions led to lower particle size supporting the hypothesis, corroborated by non published adsorption studies, that at least a part of entrapped CyA is adsorbed on PLAGA-NP surface.

CyA is a hydrophobic drug with high tendency to adsorb on many materials. Thus, a significant drug loss was suspected during NP and MS preparation. Then, CyA recovery from the samples (yield) was analyzed and its dependency on the formulation variables was established. A linearly increase of yield is obtained when the fastest injection rates and the lowest polymer amounts are used. However, when higher PLAGA amounts were used drug recovery is decreased. On the contrary, changes in needle gauge had no influence on drug loss. The highest yield value (92.06 ± 7.16%) was obtained from 147.56 mg of polymer, with 0.7 mm needle gauge and global injection rate approximately 0.200 ml/s.

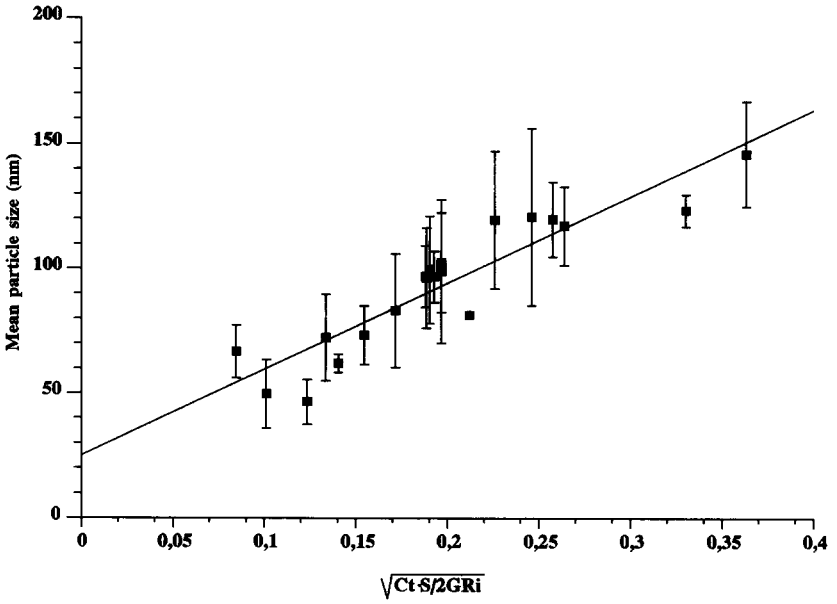

Fig. 2. Correlation between NP size and the model function for samples obtained from the central composite design. Each data point represents the mean ± SD.

Concerning MS studies, Table 4 summarizes the average results from the joint effects of the stirring rate and polymer amount on the mean size, RSD, drug encapsulation percentage and yield. The mean particle size ranged from 1.6 ± 0.05 to 30.67 ± 9.09 μm. The encapsulation percentage and the yield varied from 82.26 to 97.69% and from 77.36 to 87.80%, respectively. Yield is not affected by the variables analyzed and showed a normal distribution (Kolmogorov-Smirnov test, $p > 0.01$) with a mean value of 83.16%.

Mean particle size, RSD and encapsulation percentage can be expressed by the following polynomial equations:

$$\text{Mean size } (\mu\text{m}) = 3.1078 - 0.6439Y_1 - 9.9333Y_2 + 0.5963Y_1^2 + 6.9072Y_2^2 + 1.94Y_1Y_2$$

$$r^2 = 0.985477 \quad (6)$$

$$\text{RSD } (\%) = 19.6736 + 5.7687Y_1 - 28.0892Y_2 + 14.7145Y_2^2 - 7.6112Y_1Y_2$$

$$r^2 = 0.935286 \quad (7)$$

$$\% \text{ Encapsulation} = 89.9493 + 0.9295Y_1 - 5.3736Y_2$$

$$r^2 = 0.966924 \quad (8)$$

where Y_1 and Y_2 represent the orthogonalized values of the polymer amount and stirring rate, respectively. It can be observed from the response surface diagrams (Fig. 3a), the slower the stirring rate the larger the particle size. PLAGA amount produced a slight increase in particle size at stirring rate higher than 10 000 rpm. On the contrary, at the lowest stirring rates MS size decreases with increasing polymer concentrations. RSD (Fig. 3b), showed a similar dependency on the formulation variables as the mean size, being the stirring rate the most significant independent variable ($\alpha < 0.0001$).

These results suggest the existence of coalescence and/or aggregation phenomena during MS preparation. The coalescence is likely conditioned upon the amount of polymer due to the changes in the polymer precipitation rate. As the solvent is evaporated, the droplets become gradually con-

Table 4
Orthogonal and response values of different variables obtained from the analysis of poly D,L (lactide-glycolide) microspheres. Results are the mean of three assays determined twice

Formulation	Orthogonal values		Response values			
	Y_1	Y_2	Mean size ± SD (μm)	Relative standard deviation (%)	Encapsulation rate (%) ± SD	Yield (%) ± SD
1	−1	−1	24.74 ± 4.49	62.27	92.51 ± 2.62	84.99 ± 10.87
2	−1	1	1.68 ± 0.02	10.91	82.98 ± 5.41	82.34 ± 7.21
3	1	−1	17.24 ± 8.57	92.46	96.85 ± 1.45	84.42 ± 4.87
4	1	1	1.94 ± 0.16	10.65	85.28 ± 0.34	87.80 ± 2.27
5	0	0	3.31 ± 0.62	19.64	90.91 ± 2.73	80.49 ± 1.97
6	0	0	2.99 ± 0.44	16.04	90.35 ± 1.37	82.39 ± 5.39
7	0	0	2.94 ± 0.47	18.27	88.86 ± 7.18	77.36 ± 8.91
8	1.41421	0	4.25 ± 1.23	30.82	90.59 ± 2.49	79.65 ± 7.14
9	−1.41421	0	2.77 ± 0.70	19.35	90.09 ± 2.27	84.64 ± 10.14
10	0	1.41421	1.6 ± 0.05	12.08	82.26 ± 4.26	81.30 ± 10.02
11	0	−1.41421	30.67 ± 9.09	76.82	97.69 ± 0.78	81.64 ± 3.68
12	0	0	2.99 ± 0.38	16.85	90.04 ± 4.77	83.06 ± 5.73
13	0	0	3.51 ± 1.35	31.43	90.07 ± 2.79	87.23 ± 7.86
14	0	0	2.91 ± 0.43	15.81	90.71 ± 3.16	86.86 ± 3.37

Y_1, Polymer amount (mg); Y_2, stirring rate (rpm).

centrated and the particle nucleation takes place. The solvent evaporation rate can be considered constant in all formulations, so an increase in polymer concentration will produce particle nucleation to occur sooner. At low polymer concentrations, the droplet interphase remains flexible until the latest stages of solvent evaporation are reached and the probability of coalescence increases. Hence, the particles obtained at low stirring rate and high polymer amounts showed smaller sizes than those synthesized with lower polymer amounts. At higher agitation rates, where smaller particle size is achieved, the droplet coalescence and particle aggregation is probably caused by an insufficient surfactant amount to stabilize the system stable. The viscosity of the internal phase might also produce a significant effect on particle size since Sansdrap and Moës (1993) reported a double increase in the organic phase viscosity when the concentration of PLAGA (RG 504) increased from 2.5 to 5%. The PLAGA concentrations in this study ranged from 2.22 to 4.44%.

Zeng et al. (1994a,b) reported the same effect of stirring rate on the size of albumin microspheres prepared by high-speed homogenization (500–10 000 rpm). Similar results on particle size and CyA entrapment percentage has been notified by Sánchez et al. (1993) by using RG 503 and RG 506 polymers.

CyA entrapment percentage (Fig. 3c) showed a lineal dependency on the stirring rate and the polymer amount used to prepare the MS ($\alpha < 0.0001$). CyA encapsulation becomes higher when slower stirring rates and greater polymer amounts are used which correspond to large microparticles. In spite of this, under conditions to produce small MS a decrease in drug encapsulation was obtained because large specific surface facilitates drug diffusion to the aqueous external phase during solvent evaporation.

Electron microscopy was used in order to evaluate if shape and surface modifications on NP and MS take place by change in formulations conditions. SEM of MS is shown in Fig. 4a. They are spherical in shape, have a smooth surface and no differences were detected in their external structure due to changes in formulation. MS internal structure was observed by SEM after their inclusion into resins. It consists of a dense poly-

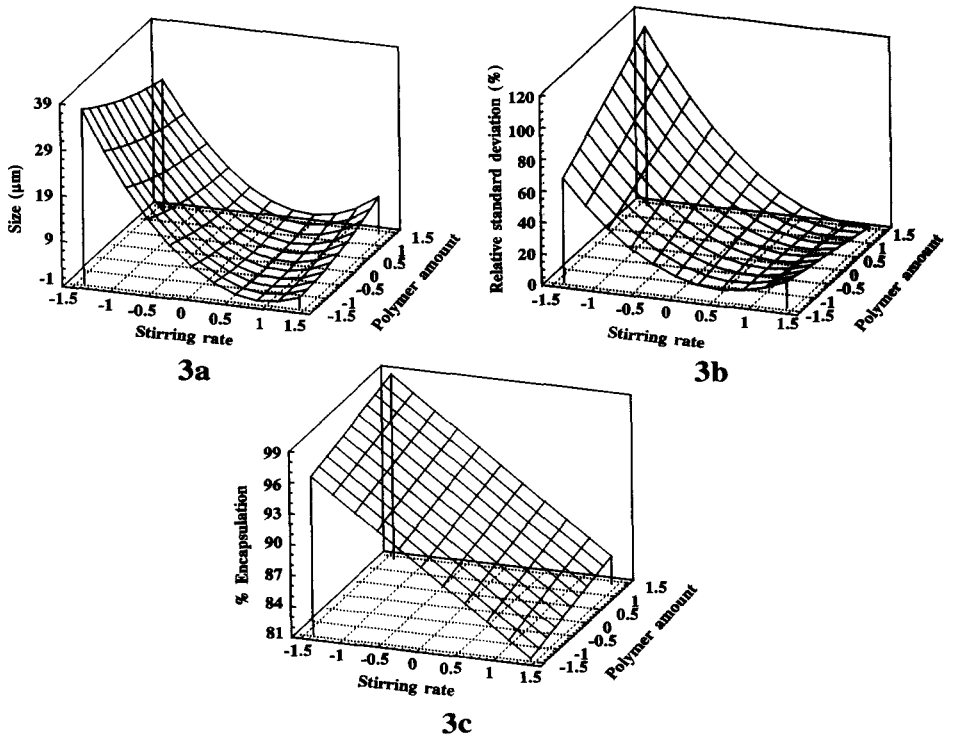

Fig. 3. Three-dimensional response surface plots showing the variation in the mean size of MS (μm) (a), the relative standard deviation (%) (b) and the CyA encapsulation percentage (c) with changes in polymer amount (Y_1) and stirring rate (Y_2).

meric matrix, where the absence of CyA crystals indicated the existence of a drug molecular dispersion that could be confirmed by differential scanning calorimetry (DSC) (Berges et al., 1995). Recently, Sánchez et al. (1993) obtained similar results, although drug concentrations in the carrier were much higher. A critical dependence of the MS porosity on the technique used for solvent removal has been reported (Jeyanthi et al., 1993; Chen and Bodmeier, 1991). In our study, the solvent evaporation takes place slowly and at constant temperature supporting the finding of dense and nonporous polymeric matrices.

Micrograph of NP observed by TEM is shown in Fig. 4b. They were also spherical and the particle diameters were in the same range as those measured by light scattering.

As a general conclusion, it is possible to easily and reproducibly elaborate CyA-loaded PLAGA NP and MS with a predetermined RSD and optimum drug encapsulation percentage and yield.

From the results here reported, two formulations for CyA oral administration have been developed and characterized. These formulations have been tested in vitro and in vivo and the results indicate PLAGA NP and MS are appropriate systems to improve in vitro CyA release and the oral bioavailability of the drug compared with a commercially available reference formulation.

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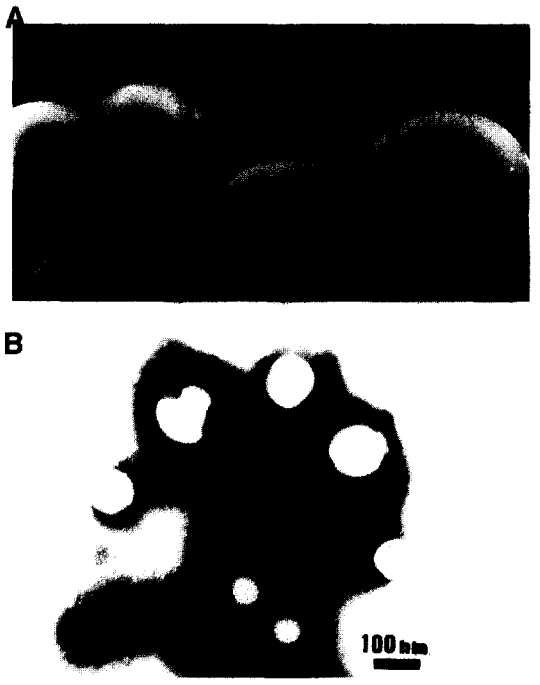

Fig. 4. Scanning electron micrograph of PLAGA MS (a) and transmission electron micrograph of PLAGA NP (b).

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