Department Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Complutense University of Madrid, Spain

Poly(ε-caprolactone) microparticles formed by the emulsion-evaporation method: effect of the variables of the different steps of the process on their size

L. Gutiérrez-Paúls, I. González-Álvarez, M. Lareo Barone, M. E. Gil-Alegre, A. I. Torres-Suárez

Received February 19, 2007, accepted March 8, 2007

Dr. A. I. Torres-Suárez, Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Complutense University of Madrid, E-28040 Spain
galaaaa@farm.ucm.es

Pharmazie 62: 864–868 (2007) *doi: 10.1691/ph.2007.11.7057*

In the present work the influence of the variables of the microencapsulation procedure on the size of poly(ε-caprolactone) microparticles (PECL-MP) obtained by the solvent evaporation method is analysed. This study will allow to choose the work conditions necessary to obtain a suitable PECL-MP size for parenteral administration. Agitation rate in the emulsion formation step, polymer concentration and organic/aqueous phase volume ratio were the variables of the microencapsulation procedure that showed a highest influence on the PECL-MP size. High polymer concentrations and low internal phase volumes had a negative effect on the microencapsulation yield. Neither the conditions of the organic solvent evaporation nor the freeze-dry process (when a cryoprotector as threalose was used) influenced on PECL-MP size. The usefulness of this study was confirmed by getting PECL-MP loaded with naloxone and with a mean diameter within 30–40 μm, suitable for parenteral administration.

1. Introduction

Poly-ε-caprolactone (PECL) is a biodegradable aliphatic polyester which is widely used as biomaterial in bioabsorbable suture material and parenteral drug delivery systems due to its high biocompatibility and its biodegradability (Sinha et al. 2004; Bezwada et al. 1995; Linhard 1989). PECL is a semicrystalline polymer with a melting point from 58 to 63 °C and a very low glass-transition temperature (−65 to −60 °C) (Middleton and Tipton 1998). It is highly soluble in organic solvents due to its hydrophobic character and its permeability is comparable to silicone rubber (Pitt et al. 1979). The degradation process of PECL is divided into two stages, the first phase involves nonenzymatic random hydrolytic cleavage of ester linkages, autocatalyzed by the carboxylic acid end groups of the polymer. During this stage the molecular weight of the polymer decreases according to a pseudo first order kinetic to less than 10% of the initial molecular weight (5.000 daltons approximately):

$$[\mathrm{COOH}] = [\mathrm{COOH}]_0 \, e^{-k't} \quad (1)$$

or

$$\mathrm{Mn} = \mathrm{Mn}^0 \, e^{-k't} \quad (2)$$

where Mn and Mn^0 are the molecular weight at times 0 and t, respectively; $[COOH]_0$ and [COOH] are the concentrations of carboxyl end groups in the polymer at times 0 and t, respectively; and $k' = k\,[H_2O]\,[E]$, where $[H_2O]$ and [E] are the concentrations of water and ester groups in the polymer, respectively. As long as the extent of chain cleavage is small, both $[H_2O]$ and [E] can be assumed constants with a value of 3.07×10^{-3} day^{-1}.

During the second stage, the diminution of the molecular weight increases and depends on the particle size. During this period, the crystallinity also increases from 45% to about 80%, due to the crystallization of tie segments. The chain cleavage in the amorphous phase, facilitated by the low glass transition temperature of PECL, makes this crystallization of tie segments possible. The crystallinity makes the access into the polymer difficult, decreasing the cleavage rate and the weight losts (Pitt et al. 1981a, 1981b). At this moment, the polymer produces a transient inflammatory response in the organism and then the PECL fragments are degraded by phagosomas of macrophages and giant cells (Schindler and Pitt 1985). The delayed degradation of PECL does not generate any acid environment, unlike other biodegradable polymers as polylactic acid and polyglicolic acid. This fact is an advantage for the development of controlled delivery systems (Dhanaraju et al. 2003; Buntner et al. 1998).

The purpose of this study was to establish a PECL microparticles elaboration guideline based on the solvent evaporation method and to evaluate the influence of the variables of the microencapsulation process on the microparticle size. This study will allow to choose the working conditions needed to obtain a specific microparticle size. The size is an important characteristic of the microparticles (MP) as drug control delivery systems for the parenteral administration for many reasons, such as, the MP must pass through an hypodermic needle for their administration; the rate of drug delivery is often strongly influenced by the system size; and the system biocompatibility also depends on the MP size, as mentioned above, due to the possibility of a tissular local reaction.

Table 1: Effect of the solvent evaporation method in the microparticles preparation and effect of the inclusion of a cryoprotector in the freeze-dry of the microparticles on the microparticles size

	Solvent evaporation method		Freeze-dried samples	
	Method I	Method II	Without threalose	With threalose
$\bar{X}$	32.83	36.23	58.49	41.53
SD_X	0.77	2.30	3.47	1.72
"t"	1.95		5.34	1.85
P	0.2342		0.0059	0.1386

$\bar{X}$: mean size (μm); SD_x: standard deviation of the mean; "t": t Student; p: probability

2. Investigations, results and discussion

2.1. Effect of the method of evaporation of the organic solvent on the characteristics of the microparticles

The results of microparticle size obtained with the two solvent evaporation methods are shown in Table 1. Significant differences were not statistically demonstrated by a "t" Student test. Neither difference in the microparticles surface was observed by electronic microscopy.
The preferable solvent evaporation method was that one under low pressure and agitation (Method II), because the elaboration time was shorter. This is an important fact when drug loaded microparticles are prepared, due to the possibility of diffusion of the active agent to the aqueous phase during the solvent evaporation (Wang and Schwendeman 1999).

2.2. Effect of the freeze-dry process on the characteristics of the microparticles

Table 1 also shows the results of the study of the influence of the freeze-dry process on the microparticles size. By means of a "t" Student test, statistically significant differences were found between microparticles size before and after the freeze-dry process when the cryoprotector was not added in the samples, whereas when threalose was used, the freeze-dry process did not modify the MP size.

2.3. Effect of the variables of the microencapsulation procedure on the characteristics of the microparticles

2.3.1. Agitation rate

Fig. 1 shows the relationship between the microparticles mean diameter and mean standard deviation with the agitation rate. As expected, MP mean diameter significantly decreased as the agitation rate increased due to the breaking-up of the internal phase of the emulsion into smaller droplets (Aberturas et al. 2002). The variation of the mean diameter and the mean standard deviation kept parallel when the agitation rate was modified.
A Multiple Range Test showed that from 2000 to 8000 rpm, there was a statistically significant inverse relationship between agitation rate and MP size, but with agitation rates higher than 8000, no significant decrease microparticle size were detected. That is, MP mean diameter decreased from 92 to 25 μm, but smaller microparticles could not be obtained even when the agitation rate was higher than 8000 rpm.
Unlike the microparticles size, the microencapsulation yield significantly improved as agitation rate increased from 8000 to 14000, so that the highest microencapsulation yield (89.9%) was obtained with the highest agitation rate tested.

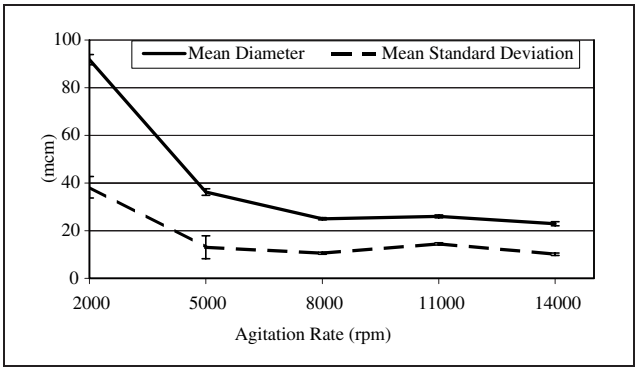

Fig. 1: Influence of the agitation rate on the microparticle size. (F_{ANOVA} = 458.35; p = 0.00)

2.3.2. Stabilizer concentration

Fig. 2 shows the evolution of the MP size with the concentration of the emulsion stabilizer. When the stabilizer concentration in the external aqueous phase increased, only a slight decrease of both the MP mean diameter and mean standard deviation was observed. But this slight variation in the MP size was statistically significant. The decrease in microparticle size is explained by the smaller droplets obtained during the emulsion formation step, due to the increase in external phase viscosity which directly depends on the concentration of stabilizer (Lin et al. 2001).
A regression analysis showed a inverse linear relationship between both variables: stabilizer concentration and microparticles size. The slope of the regression line was 8.62 μm/% what means that as stabilizer concentration increased to 1.2%, the microparticles size decreased to 10 μm.

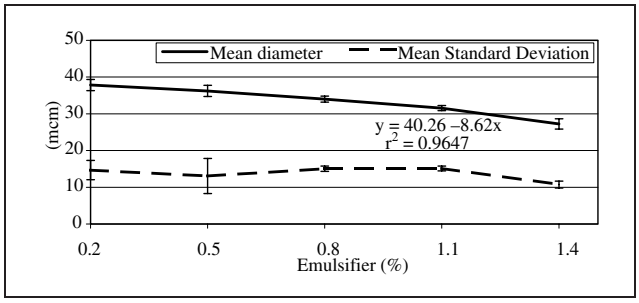

Fig. 2: Influence of the emulsifier concentration on the microparticle size (F_{ANOVA} = 9.18; p = 0.00)

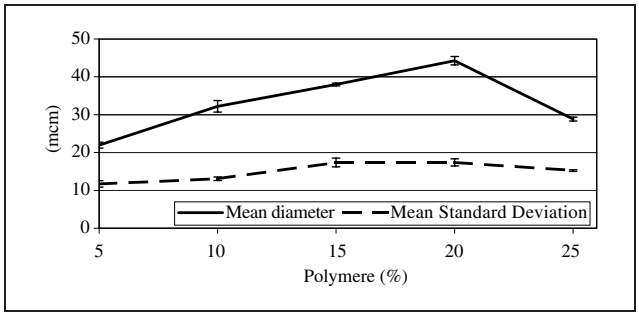

Fig. 3: Influence of the polymer concentration on the microparticle size (F_{ANOVA} = 36.74; p = 0.00)

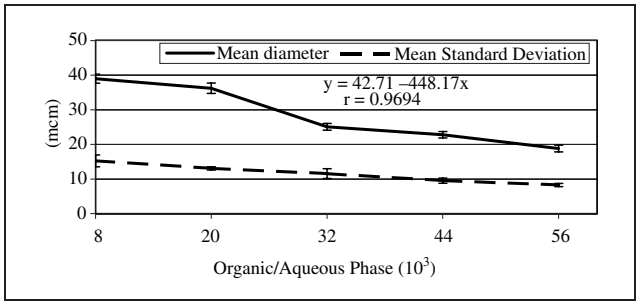

Fig. 4: Influence of the organic/aqueous phase volume ratio on the microparticle size ($F_{ANOVA} = 47.88$; $p = 0.00$)

2.3.3. Polymer concentration

Figure 3 shows the values of microparticles size obtained when the polymer concentration varied from 5% to 25%. Polymer concentration influences the internal phase viscosity, so that a higher polymer concentration would give rise to a higher viscosity of the internal phase, therefore it would be broken up in larger droplets during the emulsion formation step. Thereby, it could be expected that as polymer concentration was increased, larger microparticles were obtained (Dhanaraju et al. 2003). In fact, a linear relationship between polymer concentration and microparticle size was observed up to 20% of polymer, but with the highest polymer concentration tested, the microparticle size significantly decreased. The small microparticles size obtained with a polymer concentration of 25% may be due to the high viscosity of the internal phase making the microencapsulation process difficult: the filtration of the microparticles was a problem and abundant free polymer was observed in the supernatant. Thus, the highest value of microencapsulation yield (about 80%) was obtained when the viscosity of the internal phase was the smallest one (a polymer concentration of 5%). These microparticles fabricated from a low polymer concentration showed a less defective skin surface. This smooth surface of the microparticles has been related to a slightly tortuous matrix and a rapid drug release (Yang et al. 2001).

2.3.4. Organic/aqueous phase volume ratio

Figure 4 shows the influence of the organic/aqueous phase volume ratio on the microparticles size. When the organic/aqueous ratio increased (the volume of internal organic phase varied from 2 to 12 ml), the microparticle mean diameter statistically decreased (from 40 μm to 18.8 μm). A linear relationship was found between both variables. The standard deviation always varied as the mean diameter did.
With respect to the microencapsulation yields, when the organic/aqueous phase volume ratio was equal or greater than 32×10^{-3} (a volume of internal phase equal or greater than 8 ml), the highest microencapsulation yields, up to 80%, were obtained. On the contrary, with a organic/aqueous phase volume ratio of 8×10^{-3} (a volume of internal phase of 2 ml), microencapsulation was difficult, as proven by the low process yield obtained (about 30%), and the microparticles obtained showed a defective skin surface. In fact, when the volume of internal phase decreases, the solvent extraction towards the external phase would be facilitate and the solvent of the internal phase could be removed before the emulsion was completely formed (Kim et al. 2005).
The variables of the microencapsulation procedure, which hardly influenced on the microparticles size, were the agitation rate in the emulsion formation step, the concentration of polymer in the internal phase and the organic/aqueous phase volume ratio. As the agitation rate increased the microparticles size decreased, but an agitation rate limit was found, from which any changes in microparticles size was not detected. The higher the polymer concentration, the larger microparticles were obtained, whereas the higher organic/aqueous phase volume ratio, the smaller microparticles were obtained. Very high polymer concentrations and low internal phase volumes had a negative effect on the microencapsulation yield. The conditions of the organic solvent evaporation had not any influence on microparticle size, neither the freeze-dry process had any influence whenever a cryoprotector as threalose was used.

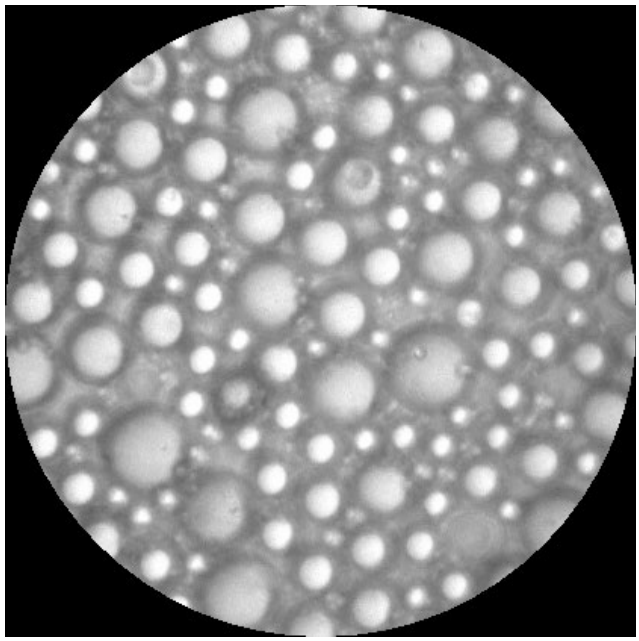

Fig. 5: Microparticles of PECL observed under an optical microscope

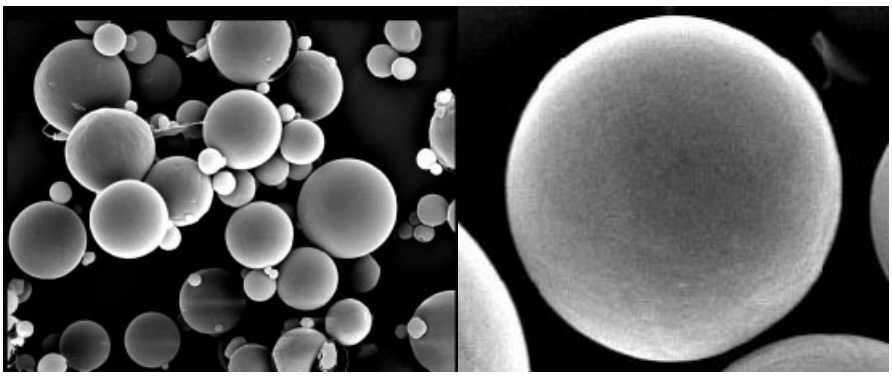

Fig. 6:
Microparticles of PECL observed under an electron microscope

Table 2: Influence of the microencapsulation variables on the characteristics of naloxone loaded microparticles

Batch	Agitation rate (rpm)	Cl_2CH_2 (ml)	MP size (μm)	SD_x (μm)	Active (%)
A	5000	5	62.12	3.21	1.22
B	8000	5	32.61	2.40	1.04
C	8000	14	24.15	1.45	3.80
D	5000	14	35.72	1.93	4.31

PVA = 0.5%; PECL = 10%

Whatever the microencapsulation protocol used, poly(ε-caprolactone) microparticles were always valid according to the objective of the present work (parenteral administration). Effectively, all the described microencapsulation methods allowed to obtain microparticles with mean sizes between 15 and 100 μm and with narrow unimodal distributions. By optic (Fig. 5) and electronic (Fig. 6) microscopy, all microparticles showed spherical shape, and did not get stuck.

2.4. Naloxone loaded nanoparticles

The knowledge of the influence of the variables studied on the characteristics of the poly(ε-caprolactone) microparticles, will allow to define the protocols of active agents microencapsulation, in order to obtain microparticles of the wanted size. With the purpose of confirming the usefulness of this study, several batches of PECL microparticles loaded with naloxone were prepared (Table 2). The aim was to achieve naloxone loaded microparticles with a mean size of 30–40 μm and high active agent content. The batch A was prepared with the fixed standard conditions of the microencapsulation procedure (section 3 under Experimental): the microparticles obtained were too much large. Then, a new batch of microparticles was prepared using a higher agitation rate (batch B): the mean size of these new microparticles was satisfactory. In order to increase the naloxone content another batch of microparticles was prepared decreasing the organic/aqueous phase volume ratio (batch C): the drug loading was trebled but, as it was expected, the microparticle mean diameter drastically decreased. To increase the mean size a last batch of microparticles was prepared decreasing the agitation rate (batch D): microparticles with the wanted characteristics were obtained.

3. Experimental

3.1. Microencapsulation procedure

Microparticles were prepared by the oil-in-water (o/w) emulsion solvent evaporation method. The internal phase was constituted by a solution of 500 mg of PECL (Sigma-Aldrich, Madrid, Spain) in 5 ml of the organic volatile solvent dichloromethane (stabilized with amilene, Panreac Chemical S.A, Barcelona, Spain). The external phase was 250 ml of a 0.5% (w/v) Polyvinyl alcohol (PVA, molecular weight: 30.000–70.000 da, Sigma-Aldrich, Madrid, Spain) aqueous solution. To form the o/w emulsion, the organic phase was incorporated into the aqueous phase, and the mixture was stirred at 5.000 rpm for 6 min (Polytron® PT 3000 electronic stirrer). Once the organic solvent was removed, the microparticles were isolated by filtering through nitrocellulose filters (pore of 5 μm) and were washed twice using 100 ml of deionized water (water purification system with an osmosis unit, Millipore S.A., Molsheim, France). Finally, the microparticles were dried at 25 °C during 24 h.
From this microencapsulation procedure, the influence of different factors on the PECL-MP characteristics is evaluated one by one. The levels of these factors are conditioned by the objective of the work: to obtain always microspheres with a suitable size for their parenteral administration.

3.2. Effect of the organic solvent evaporation method on the MP characteristics

Two different organic solvent evaporation methods were tested:
– Method I: Dichloromethane was removed at room temperature and atmospheric pressure by agitation at 200 rpm (Heidolph RZR 2051 electro-

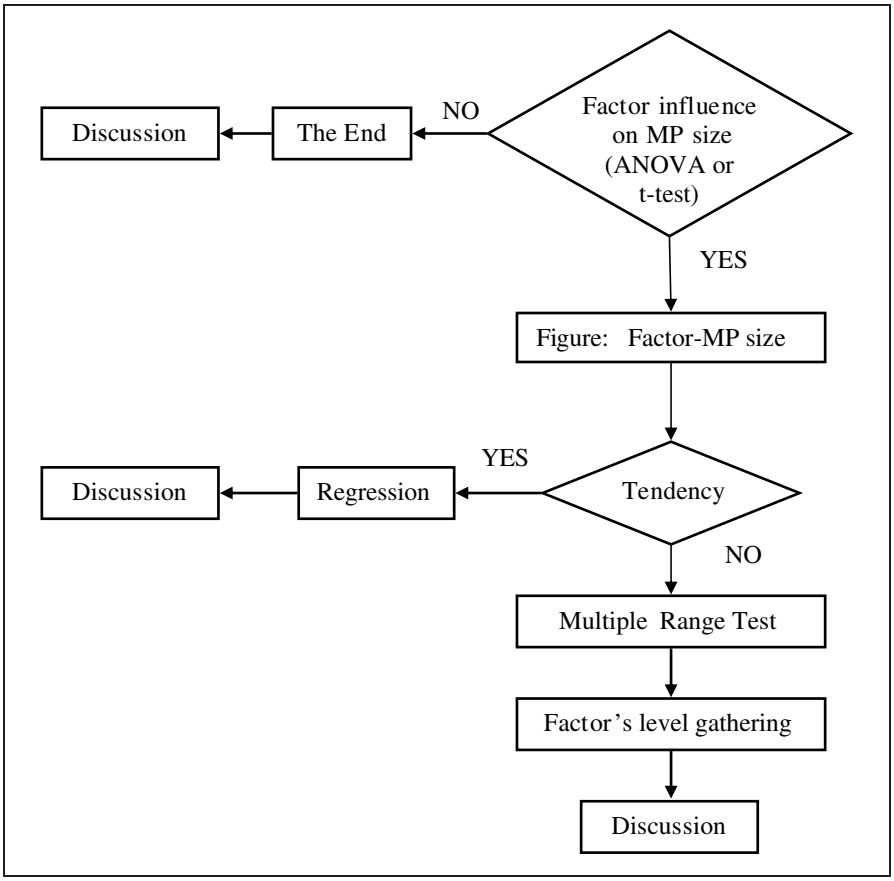

Fig. 7:
Influence of the variables of the microencapsulation process on the PECL-MP size: statistical data treatment

Table 3: Variables of the microencapsulation procedure tested

Factor	Levels				
Agitation rate (rpm)	2000	5000	8000	11000	14000
Emulsifier concentration (% w/v)	0.2	0.5	0.8	1.1	1.4
Polymer concentration (% w/v)	5	10	15	20	25
Organic/aqueous phase volume ratio	8×10^{-3}	20×10^{-3}	32×10^{-3}	44×10^{-3}	56×10^{-3}

nic stirrer) during 2 h and later by magnetic agitation for 3 h (IKA RCT basic stirrer).
– Method II: The organic solvent was removed at low atmospheric pressure using a rotatory evaporator (Vacuum Controller B-721) at 300 mm Hg during 15 min, then another 15 min at 150 mm Hg and finally 30 min at 80 mm Hg.

3.3. Effect of the freeze-drying on the characteristics of the microparticles

Samples of MP prepared using the organic solvent evaporation method II were suspended in deionized water with and without 5% (w/v) of threalose (Panreac Chemical S.A, Barcelona, Spain) as cryoprotector. Both kind of samples were instantaneously freezed with liquid nitrogen at −196 °C and were subsequently freeze-dried during 24 h. In this way, not only the effect of freeze-dry process on the MP characteristics but also the possible effect of the use of a cryoprotector were studied.

3.4. Effect of the variables of the microencapsulation procedure on the characteristics of the microparticles

The different variables tested were:
– Agitation rate to form the emulsion,
– Stabilizer concentration in the aqueous phase,
– Polymer concentration in the organic phase,
– Organic/aqueous phase volume ratio.
In the study, five different values (levels) of each variable (factors) were used (Table 3). The standard conditions were those explained previously under "Microencapsulation procedure". Different MP were prepared modifying only one variable whereas the values of the others were the standard ones. Thus, twenty batches of microparticles were prepared in triplicate.

3.5. Characterization of the microparticles

The morphology and the size of the microparticles were determined. The morphology was observed using an Olympus optic microscope and a REOL JSM 6400 electronic microscope.
Particle size was measured by laser beam diffraction, using a GALAI/CIS 1 Particle Sizer with a software which calculates the mean diameter and the standard deviation from the particle size distribution of each sample. The standard deviation was used as a indicative parameter of the size dispersion in a sample. The results of each MP batch were expressed as the mean values of the three replicates: mean diameter ($\bar{X}$), and mean standard deviation ($\bar{S}D$).
A "t" Student test (when two levels of a factor were compared) or an ANOVA (when more than two levels were compared) was carried out to demonstrate the influence of each factor tested on the microparticles size. When this influence was detected ($p < 0.05$), an appropriate statistical test was applied in order to define such influence (LSD, regression, . . .). Figure 7 shows the statistical treatment plan.

The microencapsulation process yield, calculated as the ratio between the weight of microparticles obtained and the initial PECL weight, was also determined.

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