Production of bioabsorbible nanoparticles of polycaprolactone by using a tubular recirculating system

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ABSTRACT

Encapsulation and controlled release of substances using polymeric nanoparticles require that these have a high reproducibility, homogeneity, and control over their properties, especially when they are to be used in medical, pharmaceutical or nutritional applications among others. This work shows a system for synthesizing polymeric nanoparticles that can easily control the characteristics of nanoparticles. This study shows the effect of molecular weight of the polymer, the type of surfactant and the relationship between the volumes of the aqueous and the organic phases. It was found that the first two variables have a statistically significant effect on the diameter of the nanoparticles while the relationship between the volume of the aqueous phase and the organic phase does not significantly affect the diameter of the nanoparticles. Additionally, it was found that none of the three variables significantly affects the polydispersity index (PI) of the nanoparticles.

Keywords: nanoprecipitation, delivery, nanoparticles, polycaprolactone, encapsulation.

1 INTRODUCTION

The appropriate design of encapsulation systems and controlled release systems for multiple applications in the pharmaceutical, nutrition, agricultural, textile, paints and personal care, among others, can guarantee the delivery and preservation in the short, medium and long term of different substances [1, 2]. The use of nanoparticles, specifically polymeric nanoparticles in controlled release, is a subject of increasing interest of study [3-5]. Nanoparticles of biodegradable polymers are considered important for drug delivery systems since they are very stable, easily adapt to industrial manufacturing processes over other types of nanoparticles and can be modified at their surfaces to modulate their properties [1, 6-9]. Additionally, polymeric nanoparticles can be engineered to control the rate of release of drugs as well as to control their release at a specific site in the body [9]. The modulation of their characteristics is achieved through the properties of the polymer and the chemical composition of the surface of the nanoparticles [9, 10].

To date, different methodologies for the production of polymer nanoparticles have been studied, such as nanoprecipitation, emulsion-diffusion, emulsion-coacervation, double emulsion, polymer coating, supercritical fluids and layer by layer deposition, among others [11-13]. Despite advances in each of these methodologies, the scientific community continues to face a significant challenge, namely, to guarantee the properties and reproducibility of the nanoparticles as well as to scale these systems for use in clinical trials [14, 15].

This work introduces a system for synthesizing polymeric nanoparticles. The approach can easily control the characteristics of the nanoparticles obtained and does not require specialized equipment. Additionally, the system is quite flexible and can produce small amounts of nanoparticles or it can be readily adjusted to produce large amounts. The system is based on a continuous tubular reactor that initially recirculates the aqueous phase. The organic phase is injected into the aqueous stream. The turbulence of the system in the pipe section, leads to the immediate formation of nanoparticles by the phenomenon of nanoprecipitation. The present work evaluated the flow relationship between the two currents, the molecular weight of the polymer and the influence of the surfactants in the system.

2 MATERIALS

Polycaprolactone (PCL) Mw=14.000, 45.000 and 80.000 $g \cdot mol^{-1}$, Acetone and surfactants (Pluronic F-127, Poloxamer 188 and Tween 80) were purchased from Sigma Aldrich.

3 METHODS

3.1 Recirculating system

The recirculation system is based on a continuous tubular reactor with adjustable length and tubular diameters, depending on the performance requirements. The length of the system can be varied from 30cm to 15m and the internal diameter of the reactor from 0.79mm to 38mm. For this work, the diameter of the reactor used was 3.175mm. The material of the tubular reactor used was VMQ (Vinyl Methyl-Quality) solvent-resistant silicone tubing, used in the organic phase for this study. It is recommended that materials complying with USP Class VI specifications be used. The injection of the organic phase can be modulated by changing the injection needle (18G, 20G, 21G, 22G, 23G,

25G, 27G, 29G, 30G, 31G) and modulating the injection rate, thereby controlling the particle size distribution of the nanoparticles. For this work, the 30G needle was used. Peristaltic pumps were used to control flow rates in this system.

In this system, the flow rates of the organic phase and the recirculation stream can be adjusted, varying the turbulence and the flow ratio between the two, thus controlling the size and polydispersity of the nanoparticles obtained. The recirculation system is depicted in Fig. 1.

3.2 Preparation of the nanoparticles

To prepare the nanoparticles, it was necessary use two phases. First, the organic phase was prepared containing the polymer, the solvent and the active compound to encapsulate. Subsequently, the aqueous phase was prepared with deionized water and one or more surfactants. Is possible to use some additives both phases in order to alter the properties and behavior of the final nanoparticles. Fig. 2 shows the process used to prepare the polymeric nanoparticles using the recirculating system.

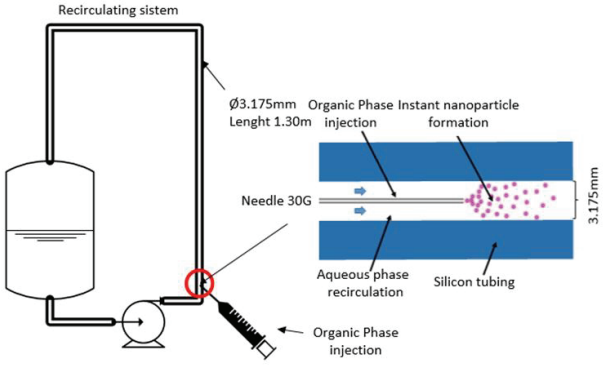

Figure 1. Description of the recirculating system

3.3 Design of experiments

In previous work carried out by the research group, it was found that the diameter of the polymer nanoparticles strongly depends on the turbulence generated in the recirculation system. For this reason, the flow rate of the recirculation system was set at 162mL·min^{-1} for a number of Re = 1249 [16]. For this work, three variables (molecular weight of the polymer, type of surfactant and ratio between the aqueous phase and the organic phase) were used, each with three level as shown in Fig. 3. The concentration of polymers in the organic phases used in this work was 4mg·mL^{-1} and the concentration of the surfactants in the aqueous phases was 0.25% w/v according to previous tests [16]. All assays were performed with 20 mL of organic phase and the volume of the aqueous phase was adjusted according to the ratio of each assay (40mL, 100mL and 200mL).

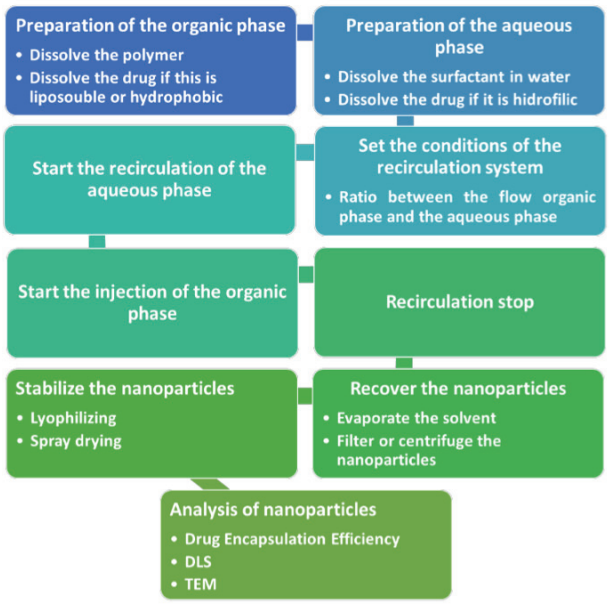

Figure 2. Preparation process of the nanoparticles

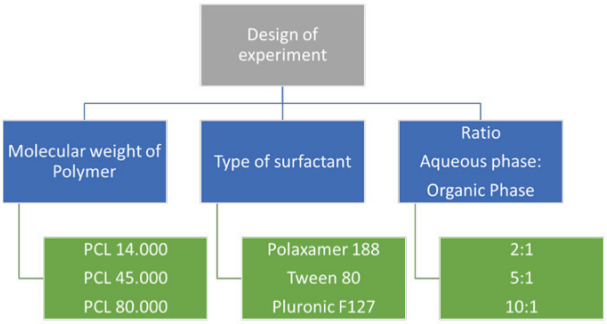

Figure 3. Design of experiment

3.4 Morphology

Transmission electron microscopy (TEM) with TEM microscope (FEI Tecnai T20) was used to study nanoparticle morphology. A droplet of the sample was mixed with a contrasting agent (1% phosphotungstic acid, pH 7.0 adjusted with 1M KOH solution) was placed on a carbon grid. The grid was pushed onto the surface of the droplet in order to create a film over the grid. The prepared sample was then placed in the TEM after 5 min for analysis.

3.5 Size and Size Distribution

The nanoparticle size and polydispersity index (PI) were evaluated by dynamic light scattering (DLS) using a Malvern Zetasizer (Nano ZS, Malvern Instruments). All measurements were carried out at 25 °C using a refractive index of 1.44 and material absorption of 0.001.

3.6 Statistical analysis

Statistical analysis of the particle size was performed applying multifactorial variance (ANOVA) using the program Statgraphics Centurion XV. Differences were considered to be significant at a level of $p < 0.05$.

4 RESULTS

4.1 Statistical analysis of DLS results

With the experimental design employed, it was possible to study the effect of the three design variables on the recirculation system and their effect on the diameter and polydispersity of the nanoparticles. The analysis of variance for the average diameter (Z_{ave}) of the nanoparticles revealed that the molecular weight of the polymer ($p = 0.0247$) and the surfactant type ($p = 0.0012$) have both $p<0.05$. Therefore, these two factors have a statistically significant effect on Z_{ave} with 95% confidence level. Conversely, the relationship between the aqueous phase and the organic phase shows a value ($p = 0.4378$). This result is important, since it indicates that in order to control the diameter of the particles, it is not necessary to work with highly diluted systems. Such a situation favors the later recovery of the nanoparticles, reducing the cost and recovery time.

From the analysis of the multiple range test for the average diameter with respect to the polymer, two homogeneous groups were found. There is no statistical difference between the results with PCL of 14.000 g·mol^{-1} and 45.000 g·mol^{-1}, nor between PCL of 45.000 g·mol^{-1} and 80.000 g·mol^{-1}. However, a significant difference was identified between the PCL of 14.000 g·mol^{-1} and 80.000 g·mol^{-1}, as can be seen in Fig. 4.

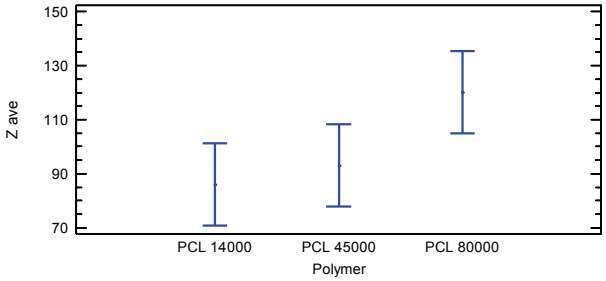

Figure 4. Means and 95% Tukey HSD Intervals for Z_{ave} and Polymers.

The results of the multiple range test for the Zave relative to the surfactant showed two homogeneous groups. There is no statistical difference between the results from Tween 80 and Pluronic F-127, but there is statistical difference between these two and Poloxamer 188, as can be seen in Fig. 5.

The results of the multiple test of ranges for the Z_{ave} regarding the relation of the aqueous phase and the organic phase revealed a homogeneous group and no statistical difference was found between the three levels evaluated. This result was expected, due to the statistical non-significance of this variable in the variance test. The results obtained can be seen in Fig. 6.

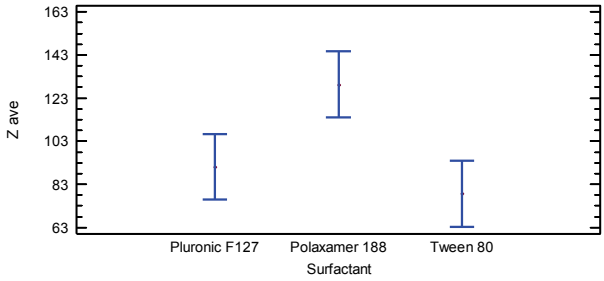

Figure 5. Means and 95% Tukey HSD Intervals for Z_{ave} and surfactants.

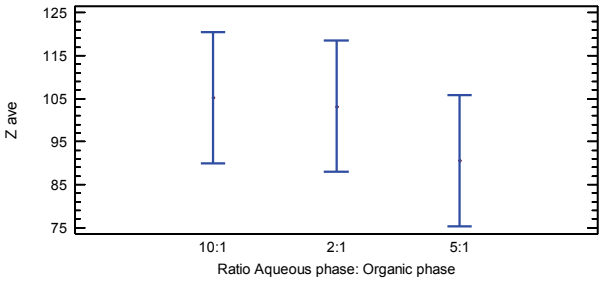

Figure 6. Means and 95% Tukey HSD Intervals for Zave and different ratios of the aqueos phase and organic phase.

From the analysis of variance on polydispersity index (PI), we found that none of the variables (molecular weight of the polymer ($p = 0.4208$), surfactant type ($p = 0.1403$), relationship between aqueous phase and organic phase $P = 0.9546$) has a significant effect on the PI response variable at the 95% confidence level. On the other hand, the average polydispersity of the system was 0.206 and the standard deviation among the tests for said variable was 0.036. These results are very significant and reinforce the results from previous studies where the index of polydispersity and diameter of the nanoparticles was found to be dependent on the turbulence in the flow of the system [16]. We should point out that in this work we used a constant flow for all the tests, decreasing the variation of polydispersity index. This further demonstrates the stability and reproducibility of the nanoparticles generated using the recirculation system.

The morphology of the PCL nanoparticles obtained was determined by TEM imaging, with well-defined spherical particles and low polydispersity index (PI <0.250). No agglomeration of the nanoparticles was observed in the analyzed images. Fig. 7. shows three TEM images as well as their corresponding histograms obtained by DLS in the Fig. 8.

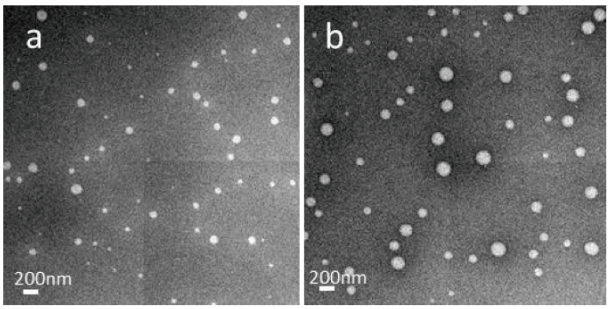

Figure. 7. DLS histogram and TEM image a) PCL 14.000 g·mol^{-1}, Poloxamer 188 and Ratio 5:1 aqueous phase and organic phase, b) PCL 45.000 g·mol^{-1}, Poloxamer 188 and Ratio 5:1 aqueous phase and organic phase.

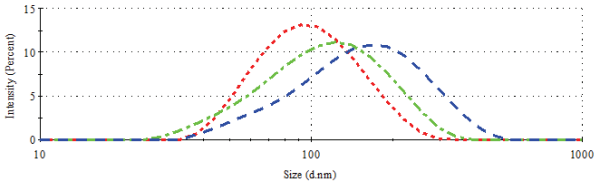

Figure. 8. DLS Frequency curve. Red) PCL 14.000 g·mol^{-1}, Poloxamer 188 and Ratio 5:1 aqueous phase and organic phase, Green) PCL 45.000 g·mol^{-1}, Poloxamer 188 and Ratio 5:1 aqueous phase and organic phase, Blue) PCL 80.000 g·mol^{-1}, Poloxamer 188 and Ratio 10:1 aqueous phase and organic phase.

5 CONCLUSION

The experimental design developed for this work showed that the molecular weight of the polymer and the type of surfactant used are relevant factors on the average diameter of the nanoparticles obtained. On the other hand, it was found that the relative proportions between the aqueous phase and the organic phase has no statistical significance on the average diameter of the nanoparticles obtained in the recirculation system developed. This is a very important result, since it demonstrates that it is possible to work with systems for nanoparticle generation that are more concentrated than commonly employed, without significantly affecting the diameter of the nanoparticles obtained. Additionally, the approach from this work allows faster, simpler and economical recovery of the nanoparticles as well as the solvent.t used.

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