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Relationship between the process variables and physicochemical features of liquid-core nanocapsules produced via nanoprecipitation

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ABSTRACT
This study investigates the relationship between the physicochemical features of liquid-core nanocapsules created via nanoprecipitation and the adopted process variables. The process variables evaluated are the amount of coating polymer (poly-ε-caprolactone), the amount of stabilizer polymer (poloxamer-188) and the stirring velocity. A statistical response surface methodology is employed to analyze the effect of the process variables on the physicochemical variables of size, polydispersity, zeta potential and efficiency in encapsulating a non-polar drug (carbamazepine). The results show that, although the process variables do not have a statistically significant effect on the response variables, the amount of coating polymer, the amount of stabilizer, and the stirring rate are physicochemically relevant. It is also found that the nanocapsules can contain more than 98% of the model drug, but the efficiency of the drug released may be affected by the process conditions.
Keywords: *Nanoprecipitation; liquid-core nanocapsules; carbamazepine; physicochemical characterization; experimental optimization*.

1. INTRODUCTION
Nanotechnology is currently identified as one of the most innovative and interesting technologies for various applications, such as pharmaceuticals, cosmetics and food science [1–12]. Several studies have demonstrated the potential of nanocapsule systems [13–17]. From a general perspective, nanocapsules can be defined as the nanovesicular systems that exhibit a typical core-shell structure, where active ingredients, such as a drug can be incorporated or confined inside an oily cavity surrounded by a polymeric or coating membrane [18,19]. Nanocapsules have been demonstrated to have many benefits over the traditional pharmaceutical dosage, such as offering better drug release kinetics [13,20], physicochemical stability [21,22] and drug bioavailability [23–25]. Moreover, their subcellular size facilitates absorption of the active pharmaceutical ingredient [26,27].
Several techniques are currently being used for nanoencapsulation and these include emulsion solvent evaporation [28–31], nanoprecipitation [13,23,32], emulsion solvent diffusion [33–37], ethanol injection [38,39] and ionic gelation [40–43]. Of these, nanoprecipitation appears to be the most straightforward and reproducible technique, as it enables highly efficient drug encapsulation in conjunction with adequate physical stability.
For this reason, nanoprecipitation is one of the most commonly used methods for preparing nanocapsules [13,23]. The technique involves several processes: *(i)* deposition of a film-forming polymer at a liquid-liquid interphase generated by dispersal of oil droplets in an aqueous solution of stabilizing polymer, *(ii)* spontaneous displacement or migration of a semi-polar solvent from the oil phase to the aqueous phase because of a gradient in surface tension (Gibbs-Marangoni effect) [17] and *(iii)* removal of the organic solvent by evaporation at a reduced pressure. The general scheme for the formation of nanocapsules is shown in Figure 1.

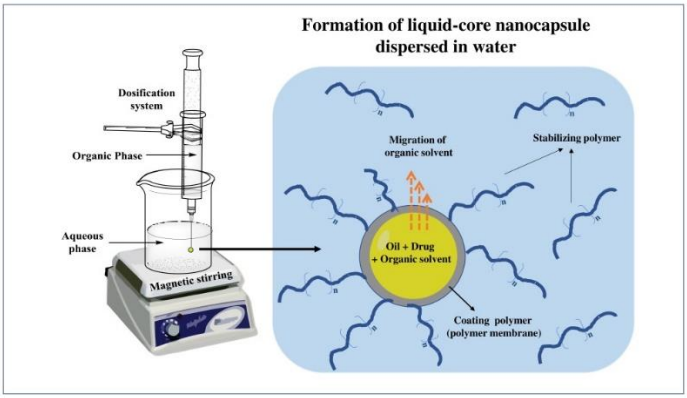

Figure 1. Set-up for the preparation of liquid-core nanocapsules through nanoprecipitation.

Although the nanoprecipitation technique has many advantages, there is not yet a consensus on the appropriate experimental conditions for this technique. Only a few studies have focused on the influence of the operational requirements on physicochemical characteristics regarding physical stability and potential performances [13,23]. In this study, we focus on evaluating the effect of the commonly used range of processing conditions on the encapsulation of non-polar drug (carbamazepine) model in the scale-up laboratory-based nanoprecipitation. The study enhances a greater understanding of the evaluation technique and its relationship with the nanoparticle characteristics, such as size, polydispersity, zeta potential and capacity for the drug encapsulation.

2. MATERIALS AND METHODS

2.1. Materials.

Carbamazepine (HPLC grade) was supplied by Tecnoquímicas S.A. (Cali, Colombia). The polymer materials of Poly-ε-caprolactone (PCL) with an average molecular weight (M_w) of 14 kDa and poloxamer-188 were from Sigma-Aldrich (St. Louis, MO, USA). Mygliol-812 was obtained from Acofarma (Terrassa, Spain). Ultra-pure water was supplied from an Elix Essential Millipore® purification system. USP-grade methanol and acetone were also purchased from Sigma-Aldrich.

2.2. Preparation of nanocapsules.

Nanocapsules loaded with carbamazepine were prepared with the nanoprecipitation method previously described by Fessi et al.[17], and two phases were prepared with the constituents listed in Table 1. 150 mg was dissolved in 4.0 mL of mygliol (oil) at 25ºC to form phase A. At the same time, the coating polymer PCL was dissolved in 25.0 mL of acetone and stirred for ~ 1 h at 500 rpm (phase B). Phase A was then dispensed onto phase B and stirred for 30 min at 500 rpm. The resulting mixture was added in a controlled manner (~1.2 ml/min) over 50 mL of aqueous solution of poloxamer-188, which instantly became 'milky' because of the formation of a heterodisperse nano-suspension corresponding to nanocapsules with oily liquid cores. Next, the acetone was eliminated by rota-evaporation (velocity: 155 rpm, temperature: 55°C, vacuum: 100 mbar, time: 40 min) until a colloidal aqueous dispersion was reached, comprising the formation of nanocapsules, and the unincorporated fraction of the drug and PCL polymer that did not create the film. Finally, the nanocapsules were purified through filtration at 100 mbar using the qualitative Whatman® filter paper (pore size: 11 µm), followed by removing the PCL aggregates retained. This procedure was repeated three times.

Table 1. Formulation proposed for the synthesis of nanocapsules loaded with carbamazepine.

Phase	Ingredients	Amount
Organic	Solvent (acetone)	25 mL
	Coating polymer (Poly-ε-caprolactone-PCL)	65-485 mg
	Miglyol	4 mL
	Carbamazepine (Drug)	150 mg
Aqueous	Non-solvent (Type II water*)	50 mL
	Stabilizer polymer (Poloxamer-188)	49-301 mg

*Obtained with an Elix Essential Millipore® purification system. Conductivity ≤ 1.0 µS/cm at 25ºC. Quantities of PCL and poloxamer were chosen according to previously reported values [13].

2.3. Optimization of experimental design.

A statistical design employing the response surfaces was used to establish whether the three process variables *(i)* amount of PCL coating polymer (x_1, mg), *(ii)* amount of poloxamer-188 stabiliser polymer (x_2, mg) and *(iii)* stirring velocity (x_3, rpm) significantly affected the following physiochemical features of nanocapsules (dependent variables): *(i)* particle size (D_H, Y_1), *(ii)* zeta potential (ζ, Y_2) and *(iii)* encapsulation efficiency (%EE, Y_3). The runs (treatments) carried out in the experiment are summarized in Table 2. A three-factor, two-level design, was selected to study the response and to construct the polynomial models that described the process. To optimize the experimental design, the central points and the set of points lying at the midpoints of each edge of the cube that defined the region of interest were replicated [44]. The experimental data from all the runs were analyzed using multiple regressions, and the differences among independent variables were determined by the analysis of variance (ANOVA). The relationship between the responses and the independent variables was visualized in contour plots developed by the Minitab software.

Table 2. Summary of the experimental design.

Treatment	Stirring velocity (rpm)	PCL (mg)	Poloxamer-188 (mg)
1	200	150	100
2	500	150	100
3	200	400	100
4	500	400	100
5	200	150	250
6	500	150	250
7	200	400	250
8	500	400	250
9	98	275	175
10	602	275	175
11	350	65	175
12	350	485	175
13	350	275	49
14	350	275	301
15	350	275	175
16	350	275	175
17	350	275	175
18	350	275	175
19	350	275	175
20	350	275	175

2.4. Determination of particle size and zeta potential.

The size, polydispersity and zeta potential of nanocapsules loaded with carbamazepine were measured by the dynamic light scattering and electrophoretic mobility, respectively, using a Zetasizer Nano Series (Malvern Instruments, Worcestershire, UK). All measurements were performed in triplicate at 25°C after an appropriate dilution (5:5000, v/v) of the nanocapsule suspension with ultra-pure water (Millipore Elix essential, Merck Darmstadt, Germany).

2.5. Determination of encapsulation efficiency.

The encapsulation efficiency (EE) was also measured in triplicate. A portion of the nanocapsule suspension was transferred to an ultrafiltration tube (VWR) with a 0.2 µm pore-size filter and centrifuged using a Hettich micro-centrifuge at an RCF of 10538. The amount of carbamazepine in the supernatant was determined by ultraviolet spectrophotometry (285 nm) using a UV spectrophotometer (Shimadzu, Kyoto, Japan). A calibration curve was obtained in 70:30 water-methanol with an R^2: 0.997. EE calculated according to the following equation:

$$EE = \frac{[Drug]_{encapsulated}}{[Drug]_{non-encapsulated} + [Drug]_{encapsulated}} \times 100$$

3. RESULTS

3.1. Preparation of nanocapsules.

The nanoprecipitation process involves the addition of one phase (organic) to another phase (aqueous) at a constant and controlled rate. Once the addition is finished, the system becomes opaque, indicating the formation of nanocapsules. Then, the particle size increases to between 1 nm and 1000 nm (nanometric scale), producing blink opalescence in the system [45].

3.2. Optimization of experimental design.

A composite factorial design, 2^3, was selected for this research. Twenty different treatments (runs) were carried out, and results regarding the response variables (particle size (D_H), zeta potential (ζ) and EE) were collected and subjected to ANOVA. The results obtained are summarized in Table 3.

Table 3. Results of analysis of variance (ANOVA).

Term	Size (nm)		Zeta potential (mV)		%EE	
	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
b_0	350.7	0.000	-27.71	0.000	----	----
b_1	26.3	0.390	2.17	0.467	99.4231	0.000
b_2	7.0	0.816	8.05	0.019	-0.0239	0.516
b_3	-11.4	0.704	-6.02	0.062	-0.1304	0.004
b_{11}	-31.3	0.528	-3.83	0.434	0.0117	0.748
b_{22}	-130.6	0.021	-2.13	0.660	-0.0546	0.369
b_{33}	-109.0	0.046	1.07	0.825	-0.0371	0.537
b_{12}	-42.3	0.526	0.07	0.991	-0.0511	0.399
b_{13}	1.4	0.983	-12.21	0.081	0.0060	0.940
b_{23}	5.8	0.930	-4.23	0.517	-0.1471	0.088
R^2	56.47%		64.25%		-0.0053	0.947
R^2-adj	17.29%		32.08%		66.02%	

b_0 = regression coefficient, b_i = linear coefficient, b_{ii} = quadratic coefficient and b_{ij} = interaction coefficient. D_H = Y1, ζ = Y2 and %EE = Y3.

A surface response methodology was implemented when a variable was influenced by other quantitative factors, to establish the values for the quantitative factors that optimized that response. For such values to occur, the independent values lead to statistically significant variation (regardless of whether this variation is meaningful, relevant or radically different). This significance was evaluated by the *p*-values obtained with ANOVA to establish two hypotheses. The first (the null hypothesis) stated that all the regression coefficients were equal to zero (showing that none of them was statistically significant). The second (the alternative hypothesis) stated that at least one of the regression coefficients did not equal zero (so at least one of them was statistically significant). To disregard the null hypothesis, the p-value for each of the coefficients must be less than the significance level (α) (in this case, α equals 0.05).

None of the *p*-values for the different coefficients in Table 3 are less than the significance level, and hence the null hypothesis cannot be disregard. Therefore, none of the independent variables (amount of PCL, amount of poloxamer-188 and stirring velocity) significantly affect the response variables (particle mean size, zeta potential and encapsulation efficiency). This conclusion is of great importance since there is no consensus on the correct method of developing nanocapsules by nanoprecipitation. The statistical result indicates that no matter which values are used for the independent variables, the results do not vary significantly, despite the observed tendencies. It is worth mentioning that the value obtained for R^2-adj for the three independent variables, (which is less than the corresponding R-value) suggests that the design is over-adjusted. These results indicate that there are variables included in the statistical designs, which are irrelevant for predicting the behavior of the dependent variables.

Although the control of the studied variables is not critical when nanoprecipitation is carried out, this does not mean that the changes do not occur in the dependent variables because of the process. Thus, it is necessary to carry out a more detailed analysis to consider and explain such differences.

3.3. Effect of the process variables on particle size and zeta potential.

To understand the carbamazepine nanoencapsulation process better, the behavior of each dependent variable as a function of the independent variables was studied while keeping the other variables constant. The effects of stirring velocity and the amount of PCL and poloxamer-188 are shown in Figure 2.

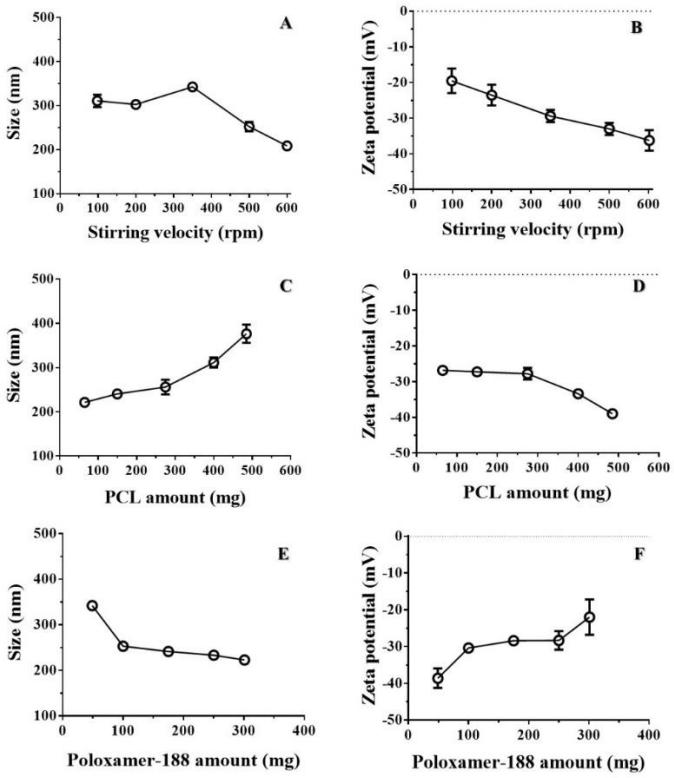

Figure 2. Effect of process variables on nanocapsule particle size and zeta potential.

3.3.1. Effect of stirring velocity. Figure 2A shows that between 98 rpm and 350 rpm, the size of the nanoparticles remains constant at ~300–350 nm. However, above 350 rpm, a decrease in size from ~350 to ~200 nm is observed. It is also important to mention that a single population with a low size polydispersity (less than 0.3) was noted in all cases. This behavior can be explained that, at high stirring velocity, there is a greater shearing effect between the miglyol droplets and the dispersing phase, leading to a reduction in the size of oil droplets. Stirring also facilitates other instantaneous phenomena and *in situ* processes, such as: *(i)* the

migration of the film-forming polymer (PCL) towards the liquid-liquid interface [46–49], and *(ii)* the migration of the organic solvent (acetone) from the oil phase to the bulk of the aqueous phase according to the Gibbs-Marangoni effect [50–53].

Figure 2B shows that the negative value of the zeta potential increases almost linearly with respect to the stirring velocity, changing from ~−20 mV to ~−35 mV. This result is very interesting, considering that the coating material (PCL) is a neutral polymer, and thus the surface of the nanocapsules should have a low zeta potential value. Such behavior could be attributed to *(i)* the formation of an anisotropic electrical double layer through the adsorption of hydroxyl ions formed by the autoprotolysis of water [54–56] or *(ii)* a possible hydrolysis effect from the esterified groups in the PCL polymer. To evaluate the latter effect, changes in zeta potential in a PCL aqueous dispersion at 350 rpm and a pH of 7.0 over 12 hours were studied. The results showed that the zeta potential remained practically constant at −20 mV, suggesting no hydrolysis in the PCL polymer. Therefore, the increase in the negative value of the zeta potential can be inferred from the greater adsorption of hydroxyl ions, which in turn, is facilitated by the increase in the surface area (or decrease in size) of the nanocapsules at such stirring velocities.

3.3.2. Effect of the amount of PCL. Figure 2C shows that particle size increases with the amount of film-forming polymer (PCL). Between 66 mg and 275 mg of PCL, a slight change in size is observed, rising from ~220 nm to ~270 nm and exhibiting low size polydispersity (less than 0.3). Between 275 mg and 500 mg of PCL, there is a more significant change in size and polydispersity, changing from ~270 nm to ~380 nm and from 0.3 to 0.6, respectively. This behavior can be explained by an increase in the viscosity of the organic phase and the formation of PCL aggregates affecting single polymer migration to the interphase zone (interfacial deposition time), thus giving the oil droplets a longer time to coalesce, increasing their size [36,57].

Figure 2D shows the zeta potential changes with an increase in the amount of PCL. Between 66 mg and 275 mg of PCL, the zeta potential is practically constant, with a value of ~−28 mV, while between 275 mg and 500 mg of PCL, there is a slight change from ~−28 mV to ~−40 mV. This result suggests that, below 275 mg of PCL, the zeta potential is controlled by the adsorption of hydroxyl ions at the interface of the nanoparticles, as explained in section 3.3.1. Above 275 mg of PCL, the formation of new systems, such as polymeric aggregates, greater adsorption of the hydroxyl ions is possible through the autoprotolysis of water. This increases the negative value of the zeta potential of the whole system (nanoparticles and polymer aggregates).

3.3.3. Effect of the amount of poloxamer-188. Figure 2E shows that between 49 mg and 100 mg of poloxamer-188, there is a decrease in particle size from 350 nm to 250 nm with a polydispersity of 0.3–0.5, indicating that the formation of several particle populations is very similar to each other. Between 100 mg and 300 mg of poloxamer-188, there is a slight decrease in size from 250 nm to 220 nm with a lower polydispersity (> 0.3). This result suggests that there is a critical concentration for achieving the steric stabilization and avoiding nanoparticle aggregation. The surfactant effect of poloxamer-188 and the Gibbs-Marangoni effect must also be considered. Regarding the Gibbs-Marangoni effect, the increase in the amount of stabiliser polymer leads to a decrease in the surface tension of the water, which generates a low gradient of superficial tension (a small gap), thus controlling the process of migration of the organic solvent (acetone) from the oily phase to the aqueous media. That is why the largest size and polydispersity are obtained at a concentration of 49 mg of poloxamer-188, as the gradient of surface tension is higher and organic solvent migration is faster, thus not providing control over the formation of nanoparticles.

Figure 2F shows a decrease in the zeta potential with respect to the amount of poloxamer-188. Between 50 mg and 100 mg of poloxamer-188, there is a change in the zeta potential from ~−40 to ~−30 mV, whereas between 100 mg and 300 mg, the negative value of the zeta potential decreases to ~−20 mV. This effect can be explained in terms of the DLVO theory [58,59], where the electrical double layer compression formed by the hydroxyl ions of the water, affecting the polarization of the nanoparticle surface and the zeta potential.

3.4. Effect on encapsulation efficiency.

All the values for carbamazepine EE were higher than 98% (Figure 3), which were in agreement with the similar results for the nanoencapsulation of non-polar drugs in nanocapsules with an oily liquid core [20–22]. It has been previously established that nanoprecipitation gives some of the best results for non-polar drug encapsulation (80% or more) [13]. Furthermore, it is widely known that some physicochemical properties of carbamazepine, especially its polarity, strongly influence the EE [44]. These results appear reasonable since this drug is highly hydrophobic (water solubility: 120 mg/L at 25°C, class II in the biopharmaceutics classification system) [25]. The EE increases as the drug increase its lipophilicity (becomes non-polar) [60–64].

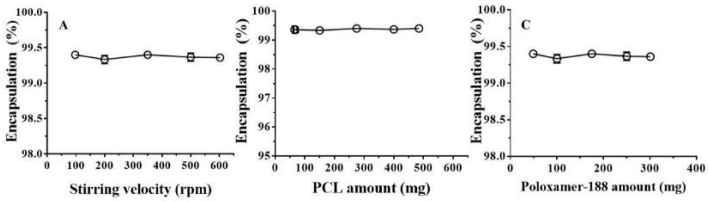

Figure 3. Effect of process variables on encapsulation efficiency.

Figure 3 shows that the EE is independent of the process variables. It has been noted in the literature that there is no relationship between the stirring velocity and EE since the drug to be encapsulated is dissolved into mygliol, and no diffusional processes are needed to achieve encapsulation [65]. It has been reported that increasing the amount of poloxamer-188 favors the dissolution of non-polar drugs in aqueous media, diminishing the drug availability in mygliol and affecting the amount encapsulated. Nevertheless, this effect was not observed in the present study.

4. CONCLUSIONS

The present study has demonstrated that nanoprecipitation is a suitable method for the preparation of nanocapsules of non-polar drugs, such as carbamazepine, capable of achieving high degrees of encapsulation. No statistically significant change in the characteristics of the nanoparticles was found to occur due to variation in the values used for the parameters of *(i)* amount of

polymer stabiliser, *(ii)* amount of film-forming polymer and *(iii)* stirring velocity when varied within the common ranges.

Some interesting physicochemical changes regarding the size and the potential zeta of the nanoparticles were observed. It was noted that an increase in the stirring velocity led to a reduction in a particle size, leading to an increase in the nanoparticle surface area. This, in turn, provides a greater area over which hydroxyl ions (from water autoprotolysis) can adhere spontaneously, forming an anisotropic electrical double layer that leads to the negative values of zeta potential between −20 mV and −40 mV.

Additionally, an increase in the amount of film-forming polymer (PCL) was shown to lead to the formation of new types of a particle with a higher number of interfaces, allowing a higher amount of hydroxyl ions to be adsorbed and thus increasing the zeta potential.

Increasing the amount of stabilizing polymer (poloxamer-188) led to a decrease in the size and the potential zeta of the nanoparticles, due to the loss of steric stabilization, favoring nanoparticle aggregation. It was further found that nanocapsules can contain more than 98% of carbamazepine, but that drug release depended on the process conditions, most critically the amount of coating polymer (PCL).

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6. ACKNOWLEDGEMENTS

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