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TRABALHO DE CONCLUSÃO DE CURSO

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Carvedilol-loaded nanocapsules: physicochemical characterization and *in vitro* drug release

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Abstract

Carvedilol (CV) has been used for the management of heart failure, hypertension and coronary artery diseases. However, it presents low oral bioavailability (25-35%). The objective of this study was to develop carvedilol-loaded nanocapsules (NC) in order to achieve a controlled drug release aiming the development of dosage forms to be administered by alternative routes. Poly(ε-caprolactone) (PCL) and Eudragit RS100 (EUD) were evaluated as polymeric wall. Nanocapsules (CV-PCL-NC and CV-EUD-NC) were prepared by interfacial deposition of preformed polymer method and characterized according to particle size and polydispersity, zeta potential, pH, drug content, encapsulation efficiency, morphology, backscattering analysis, presence of nanocrystals and drug release profile. Thermal analysis was performed to evaluate compatibility between CV and excipients. All formulations showed nanometric diameters with low polydispersity and pH slightly acid. The zeta potential was positive and negative for CV-EUD-NC and CV-PCL-NC, respectively. The drug content was close to theoretical value (0.5 $mg.mL^{-1}$) for both formulations and the encapsulation efficiency was higher than 87% and 99% for CV-EUD-NC and CV-PCL-NC, respectively. Nanocapsules were spherical-shaped and their suspensions showed no significant phenomena of physical instability. Drug release was controlled by both developed formulations. However, CV-PCL-NC showed phase separation during storage and an interaction between the drug and the surfactant was evidenced by thermal analysis. This study demonstrated the feasibility to encapsulate CV in nanocapsules to achieve a controlled release rate. Further studies will be carried out to explore alternatives routes of administration using these formulations.

Keywords: Carvedilol, nanocapsules, Eudragit RS100, poly(ε-caprolactone), drug release.

1. INTRODUCTION

Cardiovascular diseases represent an important public health problem in worldwide. They are the major cause of morbidity and mortality, and correspond to the highest costs in health care[1]. Carvedilol (CV) is a drug with a cardiovascular multiple-action. It is a non-selective β-adrenoceptor antagonist, α1-adrenoceptor blocker and also has antioxidant effects[2]. Randomized, double-bind studies have been showed that CV reduces the morbidity and mortality in patients with mild-to-severe heart failure[3-5]. The blocking effects at vascular α1 receptors, actions on vascular smooth muscle and on calcium channels contribute to its blood pressure-lowering effects; therefore this drug has been used for the treatment of hypertension[6-8]. Furthermore, CV may be useful in the management of coronary artery diseases like demonstrated in experimental models in which it showed an inhibition of the progression of atherosclerosis[2,9,10].

CV is a water-insoluble and its systemic bioavailability is just 25-35% due to its low dissolution in the intestinal tract, extensive first-pass metabolism and P-glycoprotein activity[11-14]. The encapsulation of active molecules in carrier systems in nanoscale can be a good alternative to improve the bioavailability of drugs. In this way, some formulations based on CV nanoencapsulation have been developed, such as self-nano-emulsifying drug delivery systems[15,16] and solid lipid nanoparticles[17,18]. Mahmoud et al (15), developed self-nanoemulsifying tablets of CV containing excipients with P-glycoprotein inhibition activity aiming a decrease of CV efflux and consequently an increase of its intestinal absortion. Singh et al (16) produced self-nano-emulsifying drug delivery system of CV with potential to enhance the biovailability of this drug by facilitating its transport via lymphatic circulation. Shan et al (17) prepared tablets containing carvedilol-loaded solid lipid nanoparticle that

protected CV from acid environment. They suggested that carvedilol-loaded solid lipid nanoparticles could penetrate in the lymphatic system, avoiding the first pass metabolism. Venishetty et al (18) designed carvedilol-loaded solid lipid nanoparticles and demonstrated by *in vivo* study an increase in the oral bioavailability of CV when compared with CV suspension. Another important advantage of the nanotechnology is that it allows developing innovative dosage forms to be administered by different routes of administration[19,20]. It is suggested by Saindame et al (21) the nasal route of CV administration. They developed an in situ gelling nasal spray formulation of CV nanosuspension and demonstrated by *in vivo* pharmacokinetic studies that the bioavailability of in situ nasal spray formulation was significantly increased as compared to oral administration.

Usually, medicines containing CV are administrated twice-daily for management of all diseases that it is indicated[22]. Some studies analyzed the pharmacokinetics profiles of immediate release formulations of CV administrated twice-daily and controlled-release formulations of CV administrated once-daily. Their results demonstrated similar pharmacokinetics profiles for both formulations[22,23], indicating that once-daily administration can be as effective as twice-daily administration when the drug is administrated by a controlled release approach. Moreover, the reduction of drug administration can improve patient adherence, quality of life, hospital utilization and potentially favorable effects[24]. The nanotechnology show promises on the development of systems with controlled and sustained drug-release properties[25].

Up to now, to the best of our knowledge, no reports of the encapsulation of CV in polymeric nanocapsules aimed to achieve a controlled release rate have been published. Polymeric nanocapsules are a type of nanoparticles in which the drug is confined to a cavity surrounded by a polymeric wall. They present high physical

stability and the ability to control the release of drugs[26-29]. Recently, our research group developed a new kind of nanocapsules named lipid core-nanocapsules, which are composed by a dispersion of sorbitan monostearate and medium chain triacylglycerol, in the core, surrounded by poly(ε-caprolactone) (PCL), as polymeric wall[30]. PCL offers the advantages of being a biodegradable and biocompatible polymer for application of drug delivery system[31]. Another polymer which can be used as nanocapule wall is Eudragit RS100 (EUD), a co-polymer of poly(ethylacrylate, methyl–methacrylate). It is easy to fabrication, has low cost and a good stability, representing a good material for preparation of controlled-release drug delivery systems[32,33].

In this scenario, our work aims to develop carvedilol-loaded nanocapsules prepared with two different polymers and to evaluate their potential to control the *in vitro* CV release profile, as suitable nanocarriers for the development of dosage forms for administration of CV by alternative routes of administration, like the pulmonary, nasal and subcutaneous routes.

2. METHODS

2.1. Materials

Carvedilol was obtained from Henrifarma (São Paulo, Brazil). Poly(ε-caprolactone) (MW 80,000) and sorbitan monostearate (Span 60®) were acquired from Sigma-Aldrich (São Paulo, Brazil). Eudragit RS100 was supplied from Degussa (Darmstadt, Germany) and grape seed oil from Dellaware (Porto Alegre, Brazil). Polysorbate 80 and acetone were purchase from Vetec (Rio de Janeiro, Brazil). HPLC grade acetonitrile was purchased from Tedia (Rio de Janeiro, Brazil).

2.2. Preparation of nanocapsules suspensions

Nanocapsules were prepared by interfacial deposition of preformed polymer method[34,35]. For the preparation of EUD nanocapsules (CV-EUD-NC) an organic phase was prepared dissolving 0.1 g of polymer (EUD), 165 µL of grape seed oil and 5 mg of CV (0.5 $mg.mL^{-1}$) in 27 mL of acetone under magnetic stirring at 40°C. For the preparation of PCL nanocapsules (CV-PCL-NC) the organic phase was prepared dissolving 0.1 g of polymer (PCL), 165 µL of grape seed oil, 0.0385 g of sorbitan monostearate and 5 mg of CV (0.5 $mg.ml^{-1}$) in 27 mL of acetone under magnetic stirring at 40°C. Each organic phase was injected into 53 mL of an aqueous phase containing 0.077 g of polysorbate 80 under magnetic stirring at 40°C temperature. After, acetone was eliminated and the suspension concentrated under reduced pressure (Rotavapor R-114, Buchi, Flawil, Switerzland) until final volume of 10 mL. Blank formulations were also prepared omitting the drug (BL-EUD-NC or BL-PCL-NC). The proportion of components used was based in the study of Venturi et al (35), which demonstrated the better concentration of components to obtain exclusively nanocapsules. All formulations were prepared in triplicate of batches, stored at room temperature (25 ± 2 ºC) and protected from light.

2.3. Analitycal method

Assay of CV was carried out by High Performance Liquid Chromatography (HPLC), using a method adapted from Ieggli et al (36) and validate according to our purposes. The analyzes were performed on a Shimadzu LC system (Kyoto, Japan) equipped with a CBM-20A system controller, LC-20AT pump, DGU-20A5 degasser, SIL-20A auto-sampler and a SPD-20AV. A Phenomenex Luna C_{18} column (250 mm x 4.6 mm I.D., with a particle size of 5 µm) with a C_{18} guard-analytical column was utilized. The mobile phase was composed of phosphoric acid solution pH 3.0/acetonitrile (50:50, v/v), run at a flow rate of 0.8 $mL.min^{-1}$ and detection wavelength of 241 nm. The run

time was 10 minutes. Calibration curves (n =3) were built to assay the concentration of the drug in the samples. For drug loading and encapsulation efficiency an injection volume of 10 µL was used. The method demonstrated good linearity (r = 0.9998) in the range of 1.00 - 20.00 $\mu g.mL^{-1}$. For drug release studies 20 µL of injection volume were used and it presented linearity (r = 0.9969) in the range of 0.5 – 12.5 $\mu g.mL^{-1}$. Besides, specificity was evaluated by injecting samples prepared with the blank formulations. Intraday (n=6) and interday (n=9) precision were also evaluated according to the official guidelines[37,38], showing relative standard deviation below 2.0%.

2.4. *Physicochemical characterization*

2.4.1. Particle size and polydispersity

The mean particle size of suspensions was determined by three complementary methods: laser diffraction (LD) (Mastersizer 2000, Malvern Instruments Ltd., UK), dynamic light scattering (DLS) (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK) and nanoparticle tracking analysis (NTA) (NanoSightR NTA LM 10v2, Amesbury, England). The volume-weighted mean diameters ($D_{4,3}$) and polydispersity (Span) were analyzed by LD inserting the sample directly into the compartment disperser. Mean particle size and polydispersity index (IPD) were checked by DLS after dilution of the suspensions (20 µL) in 10 mL of water previously filtered with hydrophilic membrane (0.45 µm, Millipore®). The NTA method is based on a laser illuminated microscopical technique and particles are visualized and analyzed in liquid dispersions[39]. This analysis was performed exclusively for drug-loaded nanocapsules. The CV-EUD-NC were diluted 2,500 x and CV-PCL-NC was diluted 10,000 x, both with water previously filtered with hydrophilic membrane (0.45 µm, Millipore®), and were measured for 60 s with automatic detection at room

temperature (25 ºC). For laser diffraction and dynamic light scattering the samples were analyzed in triplicate of batches (n = 3).

2.4.2 Zeta potential

Zeta potential was determined (n=3) by electrophoretic mobility on a ZetaSizer Nano ZS (Malvern Instruments Ltd., UK). The samples (20 µL) were diluted in 10 mL of 10 mM NaCl solution previously filtered with hydrophilic membrane (0.45 µm, Millipore®).

2.4.3. pH measurements

The measurement of pH was realized by potentiometry using a calibrated potentiometer (VB-10, Denver Instrument, USA) directly in the formulations. All samples were analyzed in triplicate (n = 3).

2.4.4. Transmission electron microscopy

The morphology of CV-EUD-NC and CV-PCL-NC was observed by transmission electron microscopy (TEM, Jeol JEM 1200-ExII, 100 mV, Tokyo, Japan) at Centro de Microscopia Eletrônica (UFRGS, Brazil). The samples were diluted (1:10 v/v) in purified milli-Q water, deposited on specimen grid (Formvar-Carbon support film, Electron Microscopy Sciences) and negatively stained with uranyl acetate solution (2%, w/v)[40].

2.4.5. Drug content and encapsulation efficiency

The drug was assayed by HPLC after dissolution of 1.0 mL of suspensions in 10 mL of acetonitrile followed by 10 minutes of sonication. This dispersion was centrifuged at 5000 rpm for 10 min. After, an aliquot of 2.0 mL of the supernatant was taken, diluted until 10mL in mobile phase and filtered with hydrophilic membrane (0.45 µm, Millipore®) prior HPLC analysis. Encapsulation efficiency was calculated (n = 3) by the difference between total drug and free drug. The free drug in the ultrafiltrate was assayed by HPLC after appropriate dilution with mobile phase. The ultrafiltrate was

obtained by ultrafiltration/centrifugation technique (Ultrafree-MC 10,000 MW, Millipore, Billerica, USA) at 5000 rpm during 10 min.

2.5. In vitro drug release

The *in vitro* release of CV from CV-EUD-NC and CV-PCL-NC was carried out by dialysis bag method (n = 3). Two milliliters of formulations was placed into a dialysis tubing cellulose membrane with flat width of 25mm (Sigma-Aldrich, São Paulo, Brasil) and suspended into a becker containing 100 mL of release medium (sodium phosphate buffer pH 6,8) previous filtered using a hydrophilic membrane (0.45 µm, Millipore®). The samples were maintained in a bath at 37°C under mild agitation. Sink condition was maintained during the experiment. At predetermined time intervals, 1.0 mL of the external medium was withdrawn and directly analyzed by HPLC with the method described in section 2.3.6. A CV hydroalcoholic (1:1 v/v) solution (CV-F), at the same concentration of nanocapsule suspensions (0.50 $mg.mL^{-1}$) was also prepared to evaluate the diffusion of non-encapsulated CV across the dialysis bag (n=3). In order to explore the influence of formulations on drug release profiles, the mathematical modeling mono and biexponential (MicroMath® Scientist) were used. The best model that described the drug release profile was selected through the model selection criteria (MSC), graphic adjustment and correlation coefficient. In addition, to investigate the drug release mechanism from nanocapsules it was applied the Power Law model, which allows obtaining the parameters *a* and *n*, that indicate the structural and geometric characteristics of the carriers and mechanism of release, respectively.

2.6. Multiple light scattering analysis – preliminary stability study

In order to predict the physical stability of formulations multiple light scattering analysis were carried out (Turbiscan LAbR, Formulaction, L'Union, France). This

method can evidence the tendency to occur physical destabilization phenomena (creaming, sedimentation, flocculation or coalescence) scanning the samples from the bottom to the top of the cuvette. Analyses were performed at 25 °C using 20 mL of sample without dilution, over 1 h (one scan every 5 minutes).

2.7. Differential scanning calorimetry (DSC) analysis

In order to verify possible interactions between the constituents of formulations, thermal analysis was performed using DSC equipment (TA Instruments, model Auto Q20, USA). For this, bulk CV and sorbitan monostearate and their physical mixtures (at the same proportion used in nanocapsules) were analyzed. Approximately 9 mg of samples were placed in aluminum pans and heated from 0 to 300°C with scan rates 10 °C.min^{-1} under a nitrogen atmosphere. These analysis were performed at Laboratório Multiusuário de Análise Térmica (LAMAT, UFRGS, Porto Alegre, Brazil).

2.8. Presence of drug nanocrystals

For the determination of the presence of drug nanocrystals in CV-EUD-NC, the formulation was separated in two different flasks. One flask remained motionless and at predetermined time intervals (2, 3, 5, 7 e 15 days) an aliquot was withdrawal from the surface of the suspension. The other flask was shacked for completely dispersion of particles before each sample collection, at the same time intervals of motionless flask[41]. The drug content in all samples was determined by HPLC (section 2.3.5).

2.9. Statistical analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) when three or more groups were compared and t test when two groups were compared. Difference was considered to be statistically significant at a level of $p \leq 0.05$. Data are presented as the mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Physicochemical characterization

After the preparation, all formulations showed milky bluish liquid aspect with Tyndall effect. Nanoparticles may be defined as submicron (<1μm) colloidal systems[42] and according to European Legislation (2011), products presenting 50 % of particles smaller than 100 nm can be considered as nanomaterials. Their size distributions are directly affected by qualitative and quantitative composition as well as the preparation method. For nanocapsules, one of the determining factors that influence the nanoparticle diameter is the nature of the oil that composes their core[26]. In Table 1, it can be observe that all formulations showed particle size distribution exclusively in the nanoscale range. Figure 1 shows the granulometric profiles by laser diffraction (LD) of the nanocapsules (n = 3). Independent on the presence of the drug or type of polymer, the particle size distributions are unimodal. Furthermore, analyzing the number of particles (Figure 1A), 50 % of the particles are smaller than 68 ± 2 nm, 71 ± 3 nm, 75 ± 2 and 63 ± 1 nm for BL-EUD-NC, CV-EUD-NC, BL-PCL-NC and CV-PCL-NC, respectively, being considered as nanomaterials. The volume-weighted mean diameters ($D_{4,3}$) (Figure 1B) were between 135 and 224 nm. The presence of the drug did not affect the particle size profile for EUD nanocapsules, since it was not observed significant difference ($p>0.05$) in $D_{4,3}$ between CV-EUD-NC and BL-EUD-NC. On the other hand, the presence of drug led to a significant size reduction ($p\leq0.05$) in CV-PCL-NC when compared with BL-PCL-CV. This result could indicate some interaction between components of the formulation and the drug. The polydispersity (Span) was lower than 2.0 for all formulations showing a homogenous size distribution. The hydrodynamic diameters (Z-average) and polydispersity indexes (PDI) were measured by dynamic light scattering (DLS). The mean

diameters of all formulations with EUD were similar to those determined by LD ($p>0.05$). However, by this technique we could observe a significant bigger mean diameter ($p\leq0.05$) of CV-PCL-NC in relation to the data obtained by LD. The Z-average of NC-PCL-CV was significant lower ($p\leq0.05$) than BL-PCL-NC confirming the result obtained by LD. So, there is an influence of the drug on this parameter. Polydispersity indexes determined by DLS were below 0.20, showing a narrow size distribution and confirming the results obtained by LD. In order to confirm the results of diameters obtained by LD and DLS, drug-loaded nanocapsules (CV-EUD-NC and CV-PCL-NC) were analyzed by NTA. This is an innovative technique that tracks particles in liquid suspensions through their Brownian motion, and combines the advantages of single particle (transmission electron microscopy) and ensemble (photon correlation spectroscopy) approaches[27,43]. The results of NTA were compared with the results of each technique (LD or DLS). For CV-EUD-NC, the mean diameter determined by NTA was similar ($p>0.05$) to that determined by DLS, but was significantly bigger ($p\leq0.05$) that the values obtained by LD. For CV-PCL-NC the mean diameter determined by NTA was significantly bigger ($p\leq0.05$) when compared with the other both techniques. In function of the NTA technique be based on the tracking of single particles and DLS measure a bulk of particles, it is suggested that DLS technique can be more sensible to the presence of small amounts of large particles, providing results of bigger mean diameters[44]. However, it was demonstrated by Filipe et al (45) that in some cases the intense light scattering by large particles makes the small particles more difficult to be detected and prevents some of them from being tracked by the NTA software. This phenomenon could explain the data obtained for NTA analyses for CV-PCL-NC.

The zeta potential reflects the surface potential of particles and it is affected by the polymer used as constituent of the polymeric wall, whereas it is the main component of nanocapsules. As expected, the suspensions produced with EUD as polymer exhibited positive zeta potential (Table 2). This value is coherent with the cationic nature of the polymer, which contains a quaternary ammonium group[46]. The positive charge was similar to others studies[47,48]. On the other hand, formulations with PCL showed negative zeta potential as a consequence of the non-ionic character of the polymer and the presence of polysorbate 80 at the interface particle/water, which has a negative surface density of charge due to the presence of oxygen atoms[49]. Similar results were observed in others works[50,51]. These values were significantly altered by the presence of the drug ($p \leq 0.05$). However the stabilization of these particles can be explained by a steric mechanism and not by electric repulsion, which depends on the surface charge[52]. The pH values of all formulations were slightly acid (Table 2), as other nanoparticles prepared with PCL or EUD[47,51] . The presence of the drug in nanocapsules and the type of polymer used did not influence this parameter ($p > 0.05$).

TEM micrographs demonstrate the spherical shape of nanocapsules and confirm their nanometric sizes, whose diameters were in agreement with those determined by LD, DLS and NTA (Figure 2). The drug content was close 0.5 $mg.mL^{-1}$ for both nanoparticles [0.47 ± 0.08 $mg.mL^{-1}$ for CV-EUD-NC and 0.47 ± 0.01 $mg.mL^{-1}$ for CV-PCL-NC (Figure 3)]. The encapsulation efficiency was 88 ± 1.10 % for CV-EUD-NC and 99.10 ± 0.21 % for CV-PCL-NC. Statistical analysis demonstrated that CV-PCL-NC has higher drug encapsulation efficiency than CV-EUD-NC ($p \leq 0.05$). Hence, both developed formulations of nanocapsules exhibited suitable nanoscopic

characteristics, demonstrating the feasibility of CV encapsulation in nanocapsules containing a surrounding wall formed by different polymers (EUD or PCL).

3.2. In vitro drug release

One of the advantages of nanocarrier systems is their ability to control the drug release, which is dependent to desorption of drug from the surface of the particles, diffusion through the polymeric wall, erosion of the polymer matrix or the combination of processes of diffusion and erosion[53]. In this work we analyzed the ability of EUD and PCL nanocapsules to control the CV, investigating the mechanism underlying the drug release. Results of the *in vitro* drug release study are presented in Figure 4. It can be observed that 88.49 ± 2.97 % of the CV diffused from the hidroalcoholic solution (CV-F) in 6 hours, whereas after 24 hours 73.04 ± 3.07% and 49.47 ± 2.51% of drug was released from CV-EUD-NC and CV-PCL-NC, respectively. These results demonstrated that our nanocapsules were able to retard the drug release. Moreover, nanocapsules prepared with PCL (CV-PCL-NC) showing the best control of drug release. This better performance on the drug release profile can be related with the type of the polymeric wall and/or with the presence of sorbitan monostearate in the core of CV-PCL-NC, which is a kind of nanocapsules that are composed by a lipid-core. Previous studies demonstrated that the viscosity of this kind of core increases with the presence of sorbitan monostearate and consequently decreases the drug diffusive flux[34].

The results of drug-release were analyzed by mono and biexponential mathematical modeling and the best model that described the drug release profile selected through the model selection criteria (MSC), graphic adjustment and correlation coefficient (R) was the monoexponential. Comparing the kinetic constants (k) (Table 3), that relates the drug release as a function of time, it can be observed that the drug release rate

from CV-PCL-NC was significantly slower ($p \leq 0.05$) than from CV-EUD-NC. This result confirms the difference on the drug release profile of formulations. Moreover, the mechanism of drug release was investigated using the Korsmeyer-Peppas model (Power law), which supply the release exponent (*n)* that can be used to set the release mechanism[54]. CV release from CV-PCL-NC showed regression coefficients of 0.9838 ± 0.0111, MSC of 3.13 ± 0.64 and *n* of 0.52 ± 0.07 whereas CV-EUD-NC showed regression coefficients of 0.9436 ± 0.0315, MSC of 1.86 ± 0.56 and *n* of 0.35 ± 0.01. Using this model, for spherical particles, if the release exponent *n* is equal to 0.43 a Fickian diffusion explains the release mechanism; however, if *n* has a value between 0.43 and 0.85 it indicates that the mechanism of drug release is according to an anomalous transport[54]. According to our results, the CV release from CV-PCL-NC and CV-EUD-NC can be described by two different mechanisms. For EUD nanocapsules, CV release was according to a Fickian diffusion behavior, whereas for PCL nanocapsules, CV was released by relaxation of the polymer chains as well as Fickian diffusion (anomalous transport). These results reinforce the differences of CV release when encapsulated in nanocapsules prepared with different polymers. Beyond the viscosity of the core, the more uniform arrangement of the PCL can be influencing the slower carvedilol release, because PCL has a semi-crystalline structure and this may result in more resistance to the drug diffusion and polymer relaxation[29,55].

3.3. Multiple light scattering analysis – preliminary physical stability study

When the zeta potential of nanocapsules is higher than 30 mV (in module) the charge repulsion can result in adequate stability. However, these nanostructures can be stabilized by other mechanisms than the electric repulsion, as the steric stabilization by surfactants and polymeric chains[42]. In our formulations, the surface of

the polymeric wall is covering by polysorbate 80, which is responsible for their steric stabilization[35]. In order to confirm the physical stability of the formulations, multiple light scattering analyses were carried out[56]. This technique gives information about destabilization phenomena as a function of time. In Figure 5 is possible to observe the graphical analysis of variations in the backscattering during 1 hour of analysis. No variation at the middle of the cell was observed and a small variation (< 2 %) of the delta backscattering occurred in bottom and top of cuvettes for both samples. These results show that the formulation do not present any tendency for physical instability immediately after preparation. However, about three days after the preparation of the CV-PCL-NC a visual phase separation was observed. This phenomenon was not observed for the same formulation prepared without drug (BL-PCL-NC). In order to elucidate the basis of this phenomenon, different formulations were prepared, omitting one component of the initial suspension. Two nanoemulsions omitting the polymer, containing or not the sorbitan monostearate (NE1 and NE2, respectively) were prepared, one formulation omitting the oil (nanosphere, NS) and one nanocapsule formulation using sorbitan monooleate instead of sorbitan monostearate (NC80) (Table 4).

Formulations NE1 and NS after some days of preparation showed phase separation. The phase separation was not observed for formulation NE2. These results suggested that an interaction between the drug and the sorbitan monoestearate could explain the physical instability of carvedilol-loaded PCL nanocapsules. In order to check this hypothesis, a nanocapsule formulation using sorbitan monooleate instead of sorbitan monostearate was prepared. The formulation prepared with monooleate sorbitan did not show phase separation, confirming the incompatibility between carvedilol and sorbitan monostearate. Analyzing our results we could

suggest that the interaction between sorbitan monostearate and carvedilol in CV-PCL-NC results in expulsion of the lipophilic surfactant from the core and its accumulation in the polymeric wall. As consequence of this, the interaction between the nanoparticles and water decreases a lot, resulting in the formation of two phases, water and agglomerate of nanocapsules (Figure 6). At this point it is noteworthy to mention that these physical instability is reversible, being the two phases easily redispersed after stirring. However, this limitation could be probably avoided by the conversion of the liquid suspension in dry powder materials using a drying process. Lyophilization and spray drying are strategies that have been widely studied in order to increase the stability of nanoparticle suspensions[57,58].

3.4. Differential scanning calorimetry (DSC) analysis

In order to explore the incompatibility between the components of CV-PCL-NC, which resulted in phase separation, thermal analyzes were carried out using the DSC technique. This technique provides information about physical and energetic properties of substances[61]. It is a rapid method that has been used to investigate the compatibility of materials through the appearance, shift, or disappearance of endotherms or exotherms peaks[62]. The DSC thermal curves of CV, sorbitan monosteareate and their physical mixture are showed in Figure 7. Individual components revealed an endothermic peak at 111.28 °C and 40.78 °C for CV and sorbitan monostearate, respectively. For their physical mixture, the thermal curve shows the endothermic peak of sorbitan monostearate at the same temperature (40.78°C), but the endothermic peak of CV disappeared. This result can suggest a formation of a molecular mixture of sorbitan monostearate and CV, showing a poor compatibility between these materials and confirming our previous findings about the physical instability of CV-PCL-NC containing both components. This result supports

our hypothesis of an expulsion of the molecular mixture (sorbitan monostearate + CV) from the core, resulting in the high particle aggregation followed by a phase separation. It is noteworthy to mention that this phase separation does only happened after three days of storage. All the physicochemical data and drug release data were studied using formulation prepared at the same day or in the day before the analyses.

3.5. Presence of drug nanocrystals

The process of encapsulation of lipophilic drugs in nanocapsules can result in simultaneous formation of drug nanocrystals stabilized by surfactant. These structures can be simultaneously formed when the drug concentration is higher than its saturation in the oily core. As a function of time, these nanocrystals can agglomerate and precipitate, consequently the total drug content decreases[59,41,60]. In order to verify the presence of drug nanocrystals in the developed formulations, which could have an important role on their stability and drug release profile, immobile and shaken samples were analyzed during 15 days. The drug content remained constant for both samples (immobile and shaken) after 15 days, indicating the absence of drug nanocrystals in CV-EUD-NC formulation. The presence of nanocrystals in the formulation CV-PCL-NC was not carried out because of its physical instability after three days of storage, which could interfere in the reliability of the results.

4. CONCLUSION

This study demonstrated the feasibility of CV encapsulation in nanocapsules in order control its release rate. This behavior can help in the development of dosage forms for using CV by alternative routes of administration. Nanocapsules produced with

EUD, as component of polymeric wall, showed spherical, nanometric particle size distribution and ability to control the CV release over time. Nanocapsules produced with PCL as polymer showed similar characteristics, but showed an early physical instability explained by the interaction between the drug and the sorbitan monostearate. This interaction imposes a drawback to encapsulate CV in lipid-core nanocapsules. Studies to increase the physicochemical stability of this formulation as well to explore alternatives routes of CV administration using these formulations are under development.

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Declaration of interest:

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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Table 1: Particle size distributions and polydispersity indices of Carvedilol-loaded Eudragit RS100 nanocapsules (CV-EUD-NC), Carvedilol-loaded poly(ε-caprolactone) nanocapsules (CV-PCL-NC), blank Eudragit RS100 nanocapsules (BL-EUD-NC), and blank poly(ε-caprolactone) nanocapsules (BL-PCL-NC) by laser diffraction (LD), dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA).

Formulations	LD		DLS		NTA
	D(4,3) ± SD (nm)	Span ± SD	Z-average ± SD (nm)	IPD ± DP	Mean diameter (nm)
CV-EUD-NC	135.3 ± 3.2[a]	1.21 ± 0.07	139.2 ± 5.6[a]	0.14 ± 0.005	152 ± 44
CV-PCL-NC	161.0 ± 4.0[a]	1.56 ± 0.03	179.6 ± 2.8[b]	0.08 ± 0.005	210 ± 91
BL-EUD-NC	162.0 ± 33.4[a]	1.39 ± 0.33	141.6 ± 8.8[a]	0.13 ± 0.01	-
BL-PCL-NC	224.3 ± 18.9[b]	1.69 ± 0.03	215.7 ± 8.0[c]	0.13 ± 0.02	-

SD = standard deviation (n=3). Means, in column, with the same letter are not significant different ($p > 0.05$, ANOVA).

Table 2: Zeta potential and pH of carvedilol-loaded Eudragit RS100 nanocapsules (CV-EUD-NC), Carvedilol-loaded poly(ε-caprolactone) nanocapsules (CV-PCL-NC), blank Eudragit RS100 nanocapsules (BL-EUD-NC), and blank poly(ε-caprolactone) nanocapsules (BL-PCL-NC).

Formulations	Zeta Potential ± SD (mV)	pH ± SD
CV-EUD-NC	10.65 ± 0.49[a]	6.83 ± 0.12[a]
CV-PCL-NC	-6.63 ± 0.6[b]	6.85 ± 0.03[a]
BL-EUD-NC	3.92 ± 0.8[c]	5.85 ± 0.01[a]
BL-PCL-NC	-12.22 ± 0.27[d]	6.55 ± 0.80[a]

SD = standard deviation (n=3). Means, in column, with the same letter are not significant different ($p > 0.05$, ANOVA).

Table 3: Observed rate constants (k), correlation coefficients (R) and model selection criteria (MSC) obtained by mathematic modeling of CV release from nanocarriers, Carvedilol-loaded Eudragit RS100 nanocapsules (CV-EUD-NC) and Carvedilol-loaded poly(ε-caprolactone) nanocapsules (CV-PCL-NC).

	CV-EUD-NC	CV-PCL-NC
MSC	0.6531 ± 0.1059	0.9873 ± 0.5910
R	0.9541 ± 0.0315	0.9764 ± 0.0062
k (min^{-1})	0.1170 ±0.0040[a]	0.0395 ± 0.0012[b]

Mean, in line, with different letter are significantly different ($p \leq 0.05$, t test).

Table 4: Components of formulations used to evaluate the phase separation phenomena. Nanoemulsion without sorbitan monostearate (NE1), nanoemulsion with sorbitan monostearate (NE2), nanospheres (NES) and nanocapsules with sorbitan monooleate (NC80).

Formulations	PCL	Grape seed oil	Sorbitan monostearate	Sorbitan monooleate	Carvedilol
NE1	-	165 µL	0.0385 g	-	0.005 g
NE2	-	165 µL	-	-	0.005 g
NES	0.1 g	-	0.0385 g	-	0.005 g
NC80	0.1 g	165 µL	-	0.0385 g	0.005 g

Figure1:

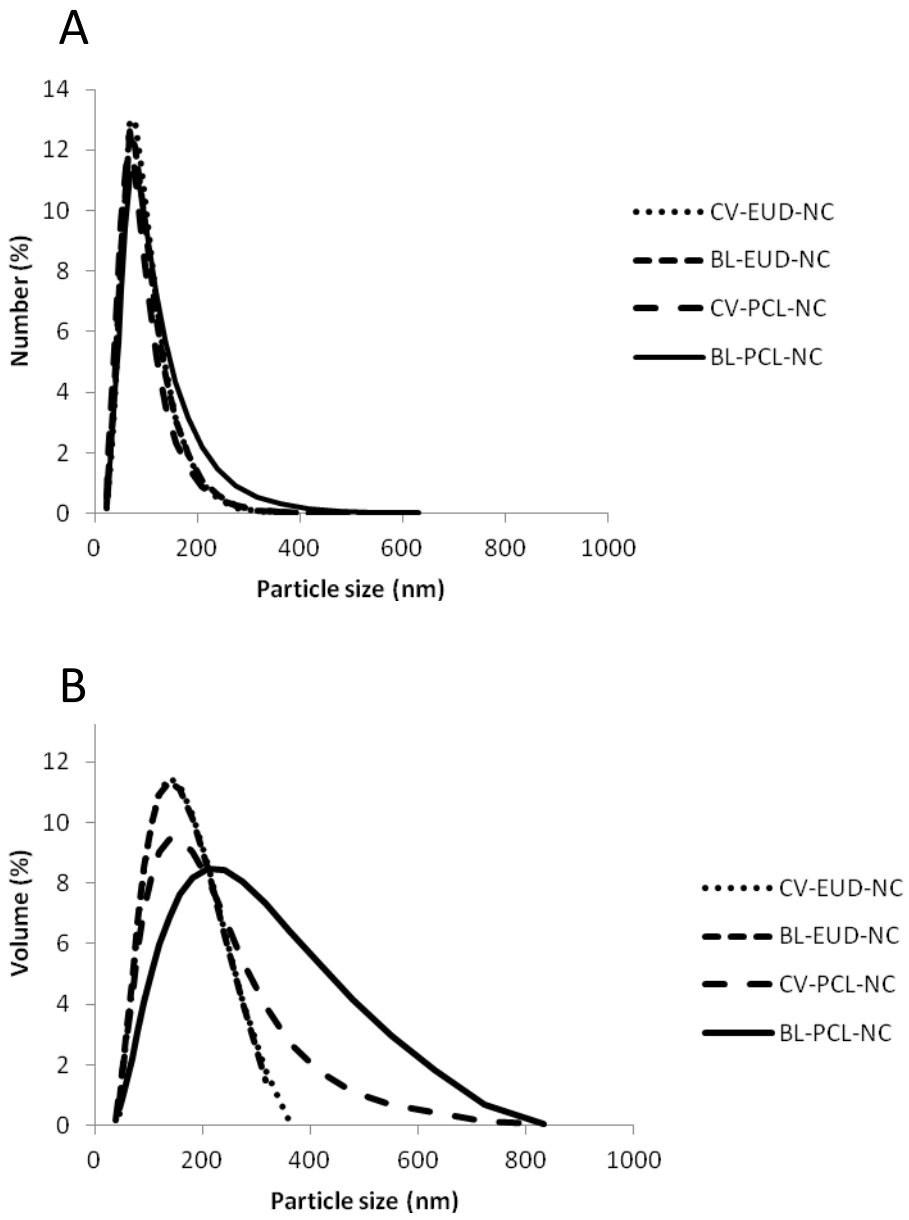

A
Number (%)
Particle size (nm)
CV-EUD-NC
BL-EUD-NC
CV-PCL-NC
BL-PCL-NC
B
Volume (%)
Particle size (nm)
CV-EUD-NC
BL-EUD-NC
CV-PCL-NC
BL-PCL-NC

Figure 2:

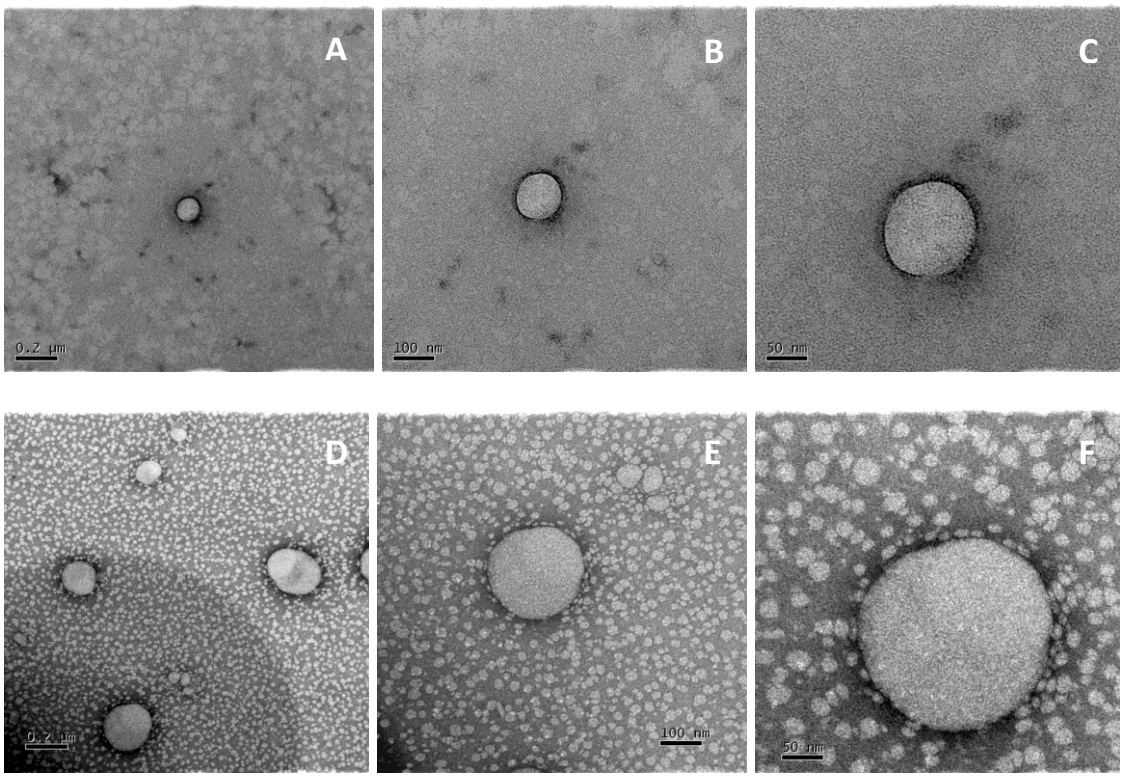
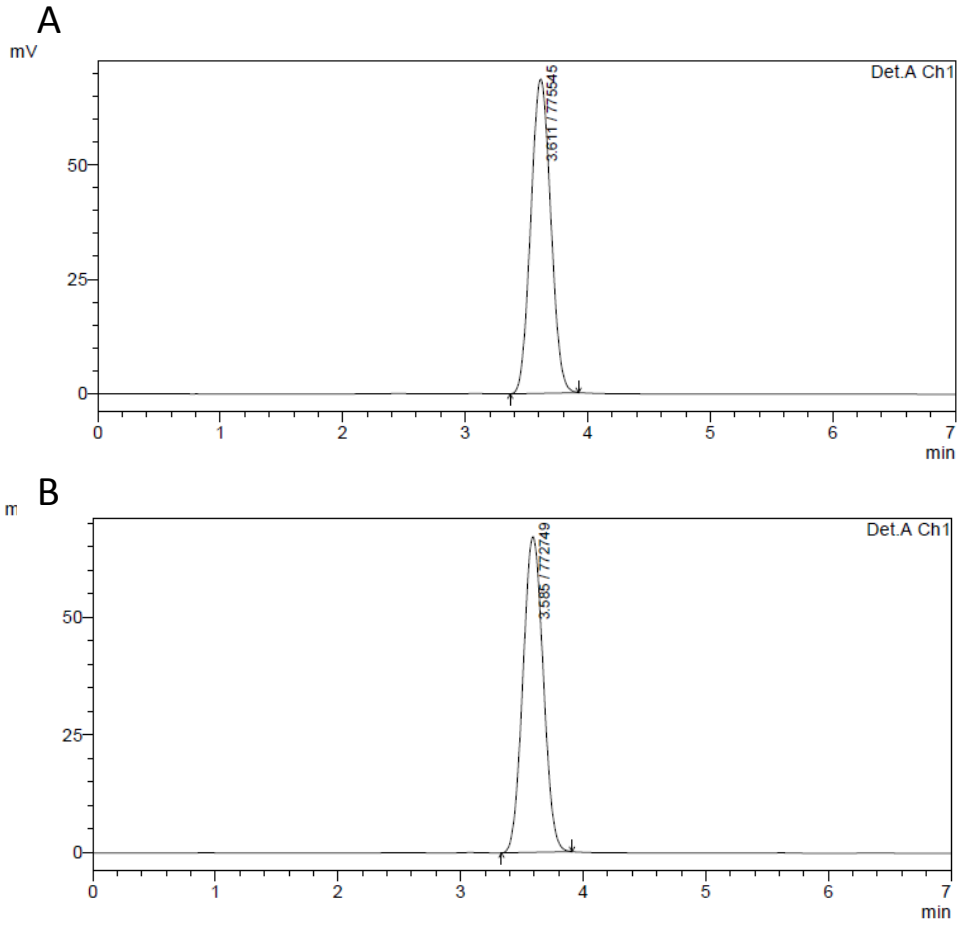


Figure 3:



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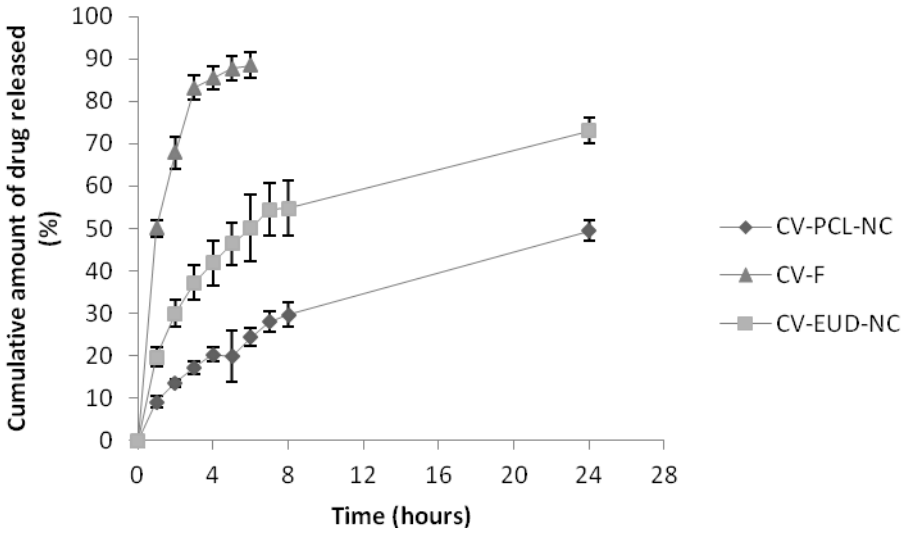

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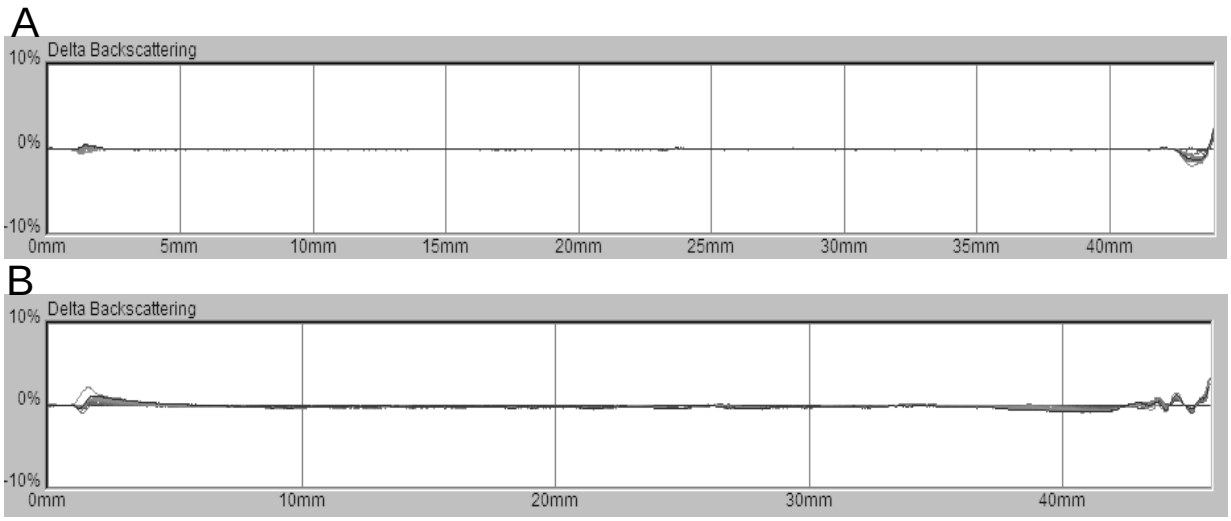

Figure 6:

Figure 7:

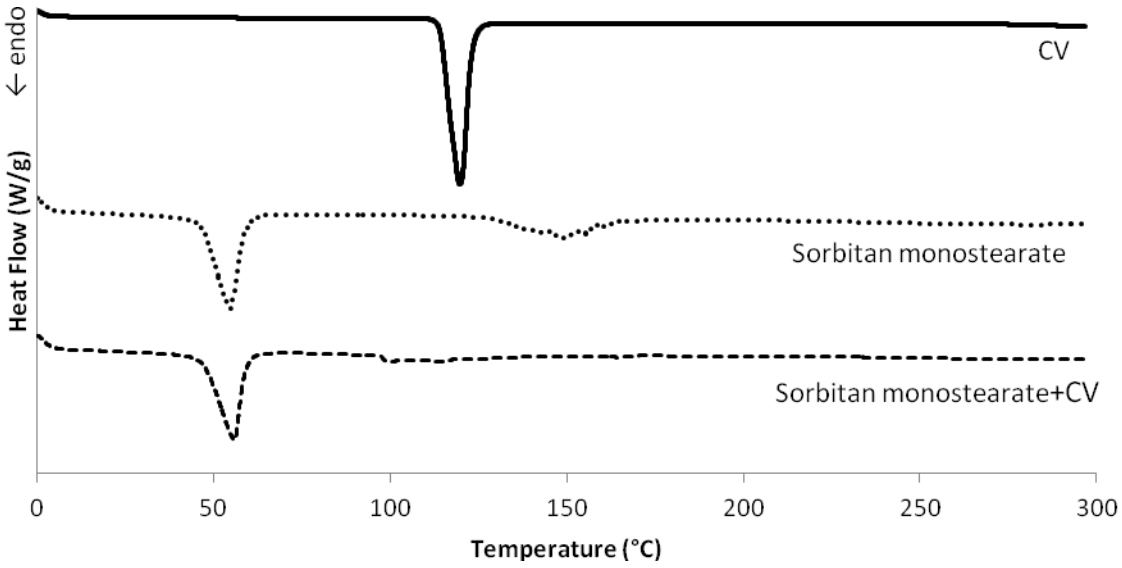
← endo
Heat Flow (W/g)
CV
Sorbitan monostearate
Sorbitan monostearate+CV
0
50
100
150
200
250
300
Temperature (°C)

Figure 1: Particle size distributions of Eudragit RS100 nanocapsules (CV-EUD-NC and BL-EUD-NC) and poly(ε-caprolactone) nanocapsules (CV-PCL-NC and BL-PCL-NC). Graphics A and B show the measurements considering number (%) and volume (%) of particles, respectively.

Figure 2: TEM micrographs: A (75000x), B (150000x) and C (300000x) CV-EUD-NC; D (75000x), E (150000x) and F (300000x) CV-PCL-NC.

Figure 3: Representative chromatograms obtained in the evaluation of drug content of carvedilol-loaded Eudragit RS100 nanocapsules (CV-EUD-NC) (A) and carvedilol-loaded poly(ε-caprolactone) nanocapsules (CV-PCL-NC) (B).

Figure 4: *In vitro* drug release profile from Eudragit RS100 nanocapsules (CV-EUD-NC) and poly(ε-caprolactone) nanocapsules (CV-PCL-NC) and from hidroalcoholic solution (CV-F) by the dialysis bag method (n = 3).

Figure 5: Delta backscattering (%) profiles of Eudragit RS100 nanocapsules (CV-EUD-NC) (A), and poly(ε-caprolactone) nanocapsules (CV-PCL-NC) (B). The bottom and top of the cuvette are displayed on the left and right sides of the images.

Figure 6: Formulation of poly(ε-caprolactone) nanocapsules (CV-PCL-NC) after phase separation.

Figure 7: Thermal curves of Carvedilol (CV), Sorbitan monostearate and their physical mixture.