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Effect of processing techniques on new poly(ε-caprolactone)-embelin microparticles of biomedical interest

Pablo R. Cortez Tornello[1] | Gabriela E. Feresin[2] | Alejandro Tapia[2] | Monika Dzieciuch[3] | Teresita R. Cuadrado[1] | Gustavo A. Abraham[1]

[1]Research Institute of Materials Science and Technology, INTEMA (UNMdP-CONICET), Mar del Plata, Argentina

[2]Instituto de Biotecnología, Facultad de Ingeniería, Universidad Nacional de San Juan, San Juan, Argentina

[3]Nanotechnology of Polymers and Biomaterials Team, Department of Chemistry, Jagiellonian University, Cracow, Poland

Correspondence
Gustavo A. Abraham, Research Institute of Materials Science and Technology, INTEMA (UNMdP-CONICET), Mar del Plata, Argentina.
Email: gabraham@fi.mdp.edu.ar

Abstract

Poly(ε-caprolactone) microparticles containing embelin (PCLE) are prepared by electrohydrodynamic atomization (EHDA) and emulsion-solvent evaporation techniques. Microparticles are characterized in terms of drug state, drug-loading capacity, encapsulation efficiency, morphology and in vitro release in different mediums. Physicochemical, morphological, and thermal characterization is presented. Kinetic analysis is performed. EHDA microparticles present higher size, surface area, and encapsulation efficiency. The release profiles combine a high initial release, followed by a slow-controlled release stage. Electrosprayed PCLE presented characteristics such as size (3.14 ± 0.05 μm), zeta potential (−49.22 ± 2.88 mV) and release profiles that could be attractive for the development of microcarriers for pulmonary administration of embelin.

KEYWORDS

electrospraying, embelin, emulsion, microparticles, poly(ε-caprolactone), release profiles

1 | INTRODUCTION

Biodegradable polymeric microparticles are being widely studied for controlled and site-specific delivery systems in pharmaceutical and tissue engineering applications.[1–3] In the development of efficient microparticle-based dosage forms, particles with good control over properties including size, porosity, morphology, surface characteristics, and drug distribution through rational particle design, are needed.[4] Microparticle manufacturing has been widely developed in the pharmaceutical industry to obtain effective drug delivery systems. There are many methods for microparticle production, being emulsion-based techniques, spray drying, hot melt, gelation, coacervation, supercritical fluid mixing, and electrohydrodynamic atomization (EHDA), the most important ones.[2,3,5,6]

Solvent-evaporation single emulsion and double emulsion methods are commonly employed to fabricate polymeric micro- and nanoparticles. However, the size distribution is broad, the process is hard to scale up, and additional difficulties associated with separation of particles from aqueous phase are also involved in the fabrication process.[7,8] On the other hand, EHDA or electrospraying is an attractive one-step method that allows the production of nearly monodisperse particles in the nano or micrometer scale. EHDA can produce particle powders without subsequent drying or separation steps, and a good control over particle size and morphology can be obtained when the processing parameters are optimized.[9–11] Scaling up for mass production is one of the most interesting advantages for many industrial applications. Zhang et al.,[12] recently described the preparation of polymeric microparticles via controlled electrostatic interactions by using multi-pore electrospraying. The modification of setups and processing parameters allows a huge variety of experimental conditions. The use of non-solvents as the collection media and variations in the deposition distance were recently reported by Gao et al.[13] as an interesting form to produce different architectures and surface morphologies. Recent advances in coaxial electrospinning allows the drug encapsulation in core/shell micro/nanoparticles. In this way, stability of drugs can be preserved and their release can be also controlled by using appropriate polymer sheaths.[14]

Electrohydrodynamic atomization is based on the atomization of liquid via strong electrostatic forces, which break up the liquid into small charged droplets that subsequently result in a homogeneous population of particles. EHDA technologies are now maturing and therefore comparisons with existing methods will become essential to further promote their utility over current methods.

Embelin, is the active constituent of the fruits of *Embelia ribes* (2.5%–3.1%), known as *Vidang* (Family Myrsinaceae). Figure 1 shows the chemical structure of embelin, which has a quite resemblance with the structure of natural Coenzyme Q10 (ubiquinones). Embelin has a wide range of pharmacological activities, including anthelmintic, antifertility, antitumor, antimicrobial, antifungal, and analgesic activities.[15–17]

Lung cancer is currently the malignant tumor with the highest mortality rate worldwide, often because it is not detected until there has been substantial progression of the illness, which leads to a significant reduction in quality of life of the patient.[18] Embelin has demonstrated antitumor activity against lung cancer cells. Recent data indicate the crucial role of p38 and JNK pathways in embelin induced apoptosis, with high selectivity and ability to inhibit XIAP (X-linked inhibitor of apoptosis protein) on cancer cells.[19] On the other hand, the effect of embelin on the sensitivity of the A549 nonsmall cell lung cancer cells to TRAIL receptor2 (TRAILR2) mAb-induced apoptosis was studied.[20]

Although many works report about the biological properties of embelin extracts and its application for the treatment of several diseases, the literature related to embelin delivery systems and its behavior in different mediums is very scarce.[21–23]

On the other hand, low water-solubility, high protein-binding, and relatively short half-life are major problems in the clinical applications of many potent anti-cancer drugs. Currently, a variety of embelin delivery systems such as liposomes,[24] nanoparticles,[25] polymeric micelles,[26,27] complexes[28] have been developed to address these problems and further to promote sustained, controlled, and targeted delivery of poorly water-soluble anti-cancer drugs.[29]

In a previous work, the authors were the first in reporting the preparation and characterization of poly(ε-caprolactone) (PCL) matrices containing embelin obtained by solution casting and electrospinning techniques.[30] PCL is a biocompatible and bioresorbable aliphatic polyester approved by U.S. Food and Drug Administration for a number of biomedical applications.[31,32] PCL exhibits a long degradation time under physiological conditions compared with other polyesters, and good drug permeability. PCL undergoes a two-stage degradation process: first, the nonenzymatic hydrolytic cleavage of ester groups, and second, when the polymer has low molecular weight (less than 3,000) the polymer is shown to undergo intracellular degradation.[32] Hydrolysis intermediates 6-hydroxyl caproic acid and acetyl coenzyme A are formed which in turn enter the citric acid cycle and are eliminated from the body.

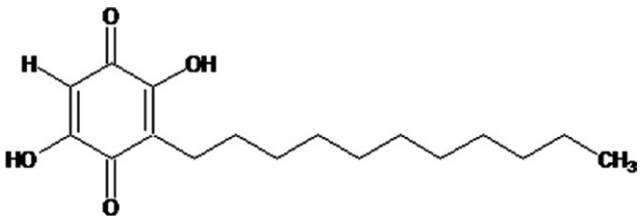

FIGURE 1 Chemical structure of embelin, a naturally occurring alkyl substituted hydroxybenzoquinone

For the first time, new systems based on PCL-embelin microparticles are prepared by electrohydrodynamic atomization and emulsion solvent evaporation techniques for the development of microcarriers for pulmonary administration of embelin. Both types of microparticles are characterized in terms of drug state, drug loading capacity, encapsulation efficiency, morphology and in vitro release in mediums with different pH. Physicochemical, morphological, and thermal characterization of microparticles are presented, and the results analyzed and compared as a function of the processing techniques. Finally, release kinetics is studied. Curves are fitted with Higuchi and Korsmeyer-Peppas models to determine the fitting parameters and its relationship with the transport and diffusion mechanisms that take place during the process. Experimental evidences, differences, and discussions regarding to how emulsion solvent evaporation and EHDA techniques affect the formation and properties of embelin-loaded PCL microparticles are presented.

2 | MATERIALS AND METHODS

2.1 | Materials

Poly(ε-caprolactone) (PCL) with a number-average molecular weight of 4.25×10^4 kg/mol. Embelin (2,5-dihy droxy-3-undecil-2,5-cyclohexadiene-1,4-dione) ($C_{17}H_{26}O_4$, Mw = 294.39 g/mol) was obtained from *Oxalis erythrorhiza* Gillies ex Hooker et Arnott (Oxalidaceae)[33,34] collected in the province of San Juan, Argentina, following the procedure described by Feresin et al.[35] Chloroform (CLF) and dichloromethane (DCM) were purchased from Aldrich Chemical Co. (St Louis, MO, USA), and used as received without further purification. Phosphate buffered saline (PBS, pH = 7.4) and phosphate-citrate buffer (PCB, pH = 5.0), poly(vinyl alcohol) (PVA), Mw = 3.1×10^4–5×10^4 g/mol and Tween 80 were also acquired from Aldrich Chemical Co.

2.2 | Methods

2.2.1 | Preparation of neat and embelin-loaded PCL microparticles by emulsion solvent evaporation technique

Neat and embelin-loaded microparticles (PCL_{em} and $PCLE_{em}$, respectively) were obtained by means of a classical

oil-in-water (o/w) emulsion technique. PCL solution 0.4% w/v was dissolved in DCM. Embelin was then added to the organic phase in a 10% w/w with respect to PCL. Emulsion was prepared by adding a PVA aqueous solution (1% or 2% w/v) or Tween 80 (0.1%, 0.2%, 0.3%, 0.4% w/v) as surfactants using a syringe pump (Activa A22 ADOX S.A., Ituzaingó, Argentina) with different infusion rates ($\Phi = 5$, 10, 15, 30 ml/hr). Homogenization was carried out with a high-performance disperser (IKA Works GmbH & Co., Germany, model Ultra-turrax® T-18) at different speeds ($v = 913$, 1772, 2912, 4193 *g*) during 1.5 hr.

The resulting emulsion was stirred during 24 hr at room temperature to facilitate solvent evaporation. Microparticles were washed three times by centrifugation during 15 min at 42857 *g* using an ultracentrifuge (Sigma GmbH 4–15, Osterode am Harz, Germany). Water was partially renewed after each cycle. Finally, water was lyophilized during 48 hr in a freeze-dryer (Virtis SP Industries, Benchtop 2.0K).

2.2.2 | Preparation of neat and embelin-loaded microparticles by EHDA technique

Poly(ε-caprolactone) solutions (7% w/v) were prepared by dissolving PCL pellets in chloroform under magnetic stirring at room temperature. For drug-loaded formulations, 10% w/w of embelin with respect to PCL was added to the polymeric solution. The flask was protected from light during the dissolution process, and the mixture was maintained under magnetic stirring at room temperature.

Each of the as-prepared solution was loaded into a standard 10 ml polypropylene syringe connected to a polyamide tube. A blunt 18-gauge stainless steel hypodermic needle was attached to the end of the tube and used as a nozzle. The flow rate was controlled by using a programmable syringe pump. A high-voltage power source (ES30P; Gamma High Voltage Research Inc., Ormond Beach, FL, USA) was used to charge the solution by attaching the emitting electrode of positive polarity to the nozzle, and the grounding one to the aluminum collecting foil. All experiments were carried out at ambient conditions (20°C and 50% relative humidity), in a chamber having a ventilation system. The solutions (typically 5–10 ml) were electrosprayed at a positive high-voltage of 17 kV, a needle-to-collector distance of 15 cm, and a solution feeding rate of 0.6 ml/hr. The aluminum foil containing the electrosprayed microparticles (PCLsp and PCLEsp) was replaced every 1 hr, dried under vacuum at room temperature to fully eliminate the residual solvent, and stored in a desiccator. Finally, the microparticles were removed to the aluminum foil using a sterile spatula and stored under vacuum in Eppendorf tubes.

Processing parameters were fixed after several experiments in which applied voltage and needle tip-to-collector distance were varied to allow a stable cone-jet regime during all the electrospraying process.

2.2.3 | Microparticle characterization

The morphology of microparticles was examined by scanning electron microscopy (SEM, JEOL JSM6460 LV, Peabody, MA, USA) at 15 kV after gold sputtering during 15 min in a chamber evacuated to 500 mTorr (Sputter coater, Desk II, Denton Vacuum, Moorestown, NJ, USA). The mean diameter and diameter distribution were obtained from the micrographs using an image-analyzer (Image-Pro Plus).

Thermal properties of PCL, embelin and embelin-loaded systems were determined by differential scanning calorimetry (DSC; PerkinElmer Inc., Model Pyris 1, Waltham, MA, USA). Scans were carried out at a heating rate of 5°C/min. Thermograms were obtained under nitrogen atmosphere. Glass transition temperature was determined in the onset of the transition. The degree of crystallinity of PCL (X_c) was calculated as:

$$X_c\,(\%) = \left(\frac{\Delta H_m \text{ experimental}}{\Delta H_m \text{ theorical}}\right) \times 100 \quad (1)$$

where the theoretical melting heat for pure high molecular weight PCL was taken as 148.05 J/g.[36]

Fourier transform infrared spectroscopy (FTIR) spectra were obtained by using a Nicolet 6700 (Thermo Scientific Inc., Waltham, MA, USA) spectrometer. Attenuated total reflectance (ATR) mode was used to record spectra over the range 4,000–450 cm^{-1} at a resolution of 2 cm^{-1}.

X-ray diffraction (XRD) patterns were taken on PANalytical X`Pert PRO diffractometer equipped with a X-ray source (Philips PW 1830, PANalytical BV) using CuKα radiation at 40 kV and 40 mA. Diffraction patterns were collected over 2θ range of 5°–75° with an acquisition time of 1 s at each step of 0.02°.

The size of drug-free and drug-loaded microparticles was measured by dynamic light scattering (DLS) (Zetasizer Nano-ZS; Malvern Instruments, Malvern, UK). Particles were transferred to an Eppendorf tube, re-dispersed in 5 ml distilled water, vortexed for 5 min before measurement, and finally transferred to a DLS cuvette. An average of three samples prepared under identical conditions was measured. Each measurement is the result of at least six runs, each one with 18 iterations. The values were expressed as the mean ± standard deviation (*SD*). The zeta potential of the different particles was determined using the same equipment.

2.2.4 | Embelin loading capacity and encapsulation efficiency

Embelin content was determined by ultraviolet-visible spectroscopy using an Agilent 8453 spectrometer (Santa Clara, CA, USA) equipped with a diode array system. A predetermined amount of sample was dissolved in DCM:DMF (1:1 by volume), and quantification was carried out after calibration

($R^2 = .989$), observing the absorption band at $\lambda = 296$ nm. At least three measurements were performed.

The loading capacity (LC) was calculated from the ratio between the embelin mass in the microparticle $(m_E)_f$ and the microparticle mass $(m_p)_f$.

$$\text{LC}\ (\%) = \frac{(m_E)_f}{(m_p)_f} \times 100 \tag{2}$$

The encapsulation efficiency (EE) was calculated as:

$$\text{EE}\ (\%) = \frac{(m_{Ef}/m_{PCLf})}{(m_{Ei}/m_{PCLi})} \times 100 \tag{3}$$

where m_{Ef} is the mass of embelin encapsulated, m_{Ei} the initial mass of embelin, and m_{PCLf} and m_{PCLi} correspond to the final and initial mass of PCL respectively. Equation (4) shows other expression used calculate the encapsulation efficiency that sometimes is reported in the literature:

$$\text{EF}\ (\%) = \frac{(m_E)_f}{(m_E)_i} \times 100 \tag{4}$$

where $(m_E)_f$ corresponds to the mass of embelin encapsulated in the microparticles and $(m_E)_i$ refers to the initial mass of embelin in the polymeric solution.

2.2.5 | Embelin release measurements

$PCLE_{em}$ and $PCLE_{sp}$ microparticles were placed in dialysis tubes (14,000 kg/mol cut-off, Sigma D9277) with 20 ml buffer solution and sealed with dialysis tubing closures. Considering that degradation process does not take place during testing, membranes with a cut-off value of 14,000 g/mol are appropriate for embelin release measurements from this kind of microparticles. Samples were then immersed in glass flasks containing 100 ml PBS pH = 7.4 or PCB pH = 5.0 at 37°C, conditions which correspond to physiological and advanced lung tumor disease respectively. Stirring was performed with an orbital shaker at 150 rpm. Samples of 1 ml were taken from the dissolution medium each 1 hr during the first 12 hr, and one sample per day was taken during 6 days. After each extraction, 1 ml of fresh solvent was added. The total volume extracted was lower than the 15% of the total solution volume. All the assays were done in triplicate. The concentration of embelin was determined by UV–Vis spectroscopy measuring the absorption band at $\lambda = 330$ nm for PBS and $\lambda = 349$ nm for PCB. The percentage of embelin released was calculated by dividing the cumulative amount of embelin released at each sampling time by the initial amount of embelin $(m_E)_i$.

2.2.6 | Release kinetic studies

In order to study the release mechanisms, Higuchi and Korsmeyer-Peppas models, two mathematical models commonly used to describe drug-release profiles, were used to fit the experimental data using nonlinear regression. The results were compared in terms of goodness of the fit.

The Higuchi model (Equation 5) was initially conceived for planar systems, and then extended to different geometries.[37] This model has many assumptions, including perfect sink conditions throughout the experiment, initial drug concentration higher than drug solubility, and constant drug diffusivity[38]:

$$\frac{M_t}{M_\infty} = A + K_H \cdot t^{1/2} \tag{5}$$

where M_t/M_∞ is the fractional release of drug at time t (M_t and M_∞ are the amount of drug released at time t, and at infinite time, respectively), K_H is the kinetic constant that include design characteristics of the system, and A is a constant that represents the extension of the burst release.

The Korsmeyer-Peppas model (Equation 6), known as power law's model, is also widely used for its simplicity to fit the first 60% of the release data:

$$\frac{M_t}{M_\infty} = A + K_{KP} \cdot t^n \tag{6}$$

where K_{KP} is the kinetic constant that include design characteristics of the system, A is a constant that represents the extension of the burst release, and n is the release exponent, that characterize the mechanism of drug release.

2.2.7 | Statistical analysis

All the assays were performed in triplicate, and the data are expressed as mean ± *SD*. The statistical differences were determined by applying the student's test with a confidence interval of 95% ($p \leq .5$ and $n < 30$).

$$t = \frac{(x - \bar{x})}{(S/\sqrt{n})} \tag{7}$$

where t is the experimental value of the Student variable, x is the experimental data value, $\bar{x}$ is the mean data value, S is the standard deviation, and n is the number of measurements.

3 | RESULTS AND DISCUSSION

3.1 | Physicochemical and morphological characterization of microparticles

Polymeric microparticles were successfully prepared from PCL, by means of emulsion solvent evaporation and EHDA techniques. In order to determine the best processing conditions for microparticle formation, several experiments using different parameters were carried out.

The morphology of samples obtained by emulsion solvent evaporation using PVA (1% wt/v) was studied by SEM as

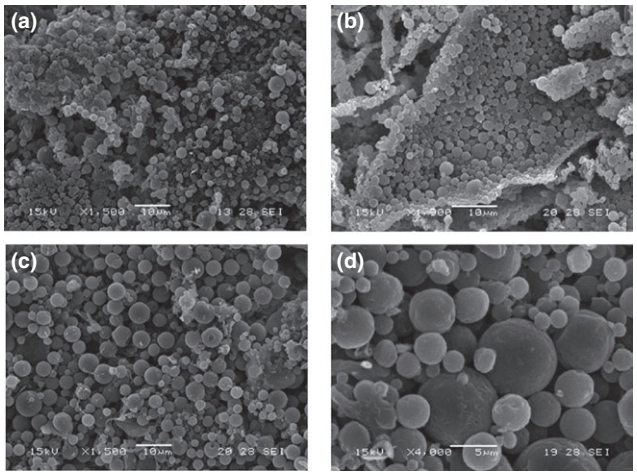

FIGURE 2 SEM micrographs (1,500×) of PCL microparticles obtained by emulsion solvent evaporation using PVA 1% wt/v: (a) v = 15,600 rpm, Φ = 10 ml/hr; (b) v = 24,000 rpm, Φ = 5 ml/hr; (c) embelin-loaded microparticles obtained with v = 24,000 rpm, Φ = 10 ml/hr; (d) PCL microparticles obtained with Φ = 10 ml/hr and v = 24,000 rpm. PCL, poly(ε-caprolactone); PVA, poly(vinyl alcohol)

a function of the processing parameters. Figure 2 shows the relevant images.

The effect of different infusion rate and dispersion speed on the microparticles quality and shape is shown in Figure 2. The microparticle formation was affected by these parameters, and among the explored one, the most favorable were Φ = 10 ml/hr and v = 1772 g (Figure 2a). Then, the range of infusion rate and dispersion speed values was extended. Lower infusion rate (Φ = 5, 10 ml/hr) and higher homogenization speed (v = 20,000, 24,000 rpm) were used. Figure 2b revealed that homogeneous and disaggregated microparticles were obtained when a dispersion rate of 24,000 rpm was used. Based on these experimental conditions, embelin-loaded microparticles were prepared. Microparticles of $PCLE_{em}$ and PCL_{em} obtained with v = 24,000 rpm and Φ = 10 ml/hr (Figure 2c,d, respectively) presented low aggregation and narrow particle size distribution.

In the emulsion process, type, composition, and concentration of surfactants affect surface properties. Particle stability is strongly affected by surface composition. In order to increase stability and decrease the agglomeration degree, different concentrations of PVA and Tween 80 were used. PVA (1% w/v) allowed the formation of spherical microparticles with high stability (Figure 2) whereas with the use of Tween 80 the particles had irregular shape. The use of 2% w/v of PVA led to higher particle agglomeration.

The morphology of microparticles produced by EHDA (PCL_{sp} and $PCLE_{sp}$) was studied by SEM (Figure 3). PCL_{sp} presented a defined spherical shape with certain aggregation degree (micrograph 3A), whereas the encapsulation of embelin in $PCLE_{sp}$ led to less agglomerated particles with non-smooth surface (micrograph 3B). The observed rough surface morphology contributes to avoid microparticle aggregation. This result agrees with the reported by Xie et al.[39] They found that high paclitaxel loading helped in the formation of non-agglomerated particles. The drug/polymer mass ratio, solvent evaporation rate and polymer diffusion rate played an important role during the particle formation process and the resulting morphology.[40] Solvent evaporation from the droplets involves a combination of heat and mass transfer process. Low-boiling point solvents (e.g., DCM, 40°C) have fast evaporation rates and could facilitate the formation of particles with porous or hollow structures. Conversely, solvent with high boiling points (e.g., DMF, 152°C) could result in incomplete evaporation and partial dissolution of semi-solid particles once they reach the collector. Therefore, solvents having intermediate boiling point as CLF (61°C) could be responsible for the formation of particles with rough or partially smooth surfaces.[41] The morphology observed in $PCLE_{sp}$ indicates the high surface area of these particles could be very useful for delivery of therapeutics in pulmonary applications particularly in systemic inhalation therapies.[42]

Table 1 shows the particle size, polydispersity index (PDI), and zeta potential values. PCL_{sp} and $PCLE_{sp}$ microparticles exhibited higher mean diameter than PCL_{em} and $PCLE_{em}$. Embelin encapsulation led to an increase in particle size. This observation is in agreement with the reported by Enayati et al.[43] for PCL particles incorporating β-oestradiol. However, other authors found the opposite trend, a smaller sized with increasing drug content

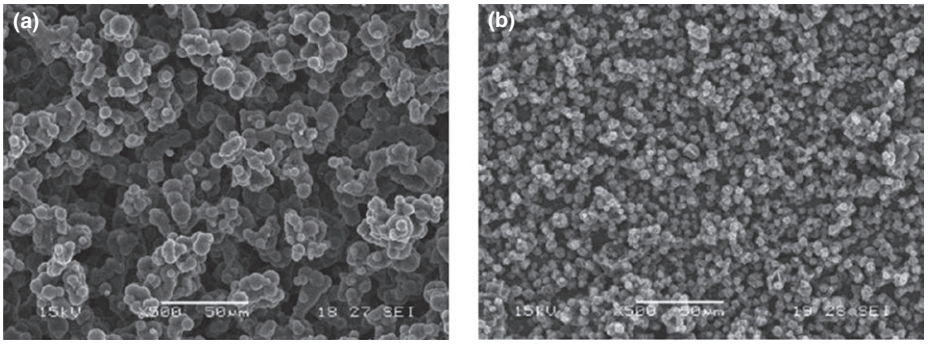

FIGURE 3 SEM micrographs of PCL microparticles (500×): (a) PCL_{sp}; (b) $PCLE_{sp}$. PCL, poly(ε-caprolactone)

TABLE 1 Mean diameter (D_m) and zeta potential of microparticles and embelin content incorporated in PCLE microparticles

Sample	D_m (μm)	PDI	Zeta potential (mV)	ME (mg/g)	LC (%)	EE (%)
PCL_{em}	1.68 ± 0.07	0.05 ± 0.020	−15.80 ± 0.49	—	—	—
PCL_{sp}	2.28 ± 0.11	0.06 ± 0.011	−1.74 ± 0.12	—	—	—
$PCLE_{em}$	2.72 ± 0.13	0.06 ± 0.005	−34.10 ± 2.40	10.54 ± 0.89	1.05 ± 0.14	10.65 ± 0.01
$PCLE_{sp}$	3.14 ± 0.05	0.03 ± 0.006	−49.22 ± 2.88	60.81 ± 0.79	6.08 ± 0.13	64.75 ± 0.01

ME, mass of embelin per mass unit of sample; LC, loading capacity; EE, encapsulation efficiency calculated according to Equation (3), $n = 3$.

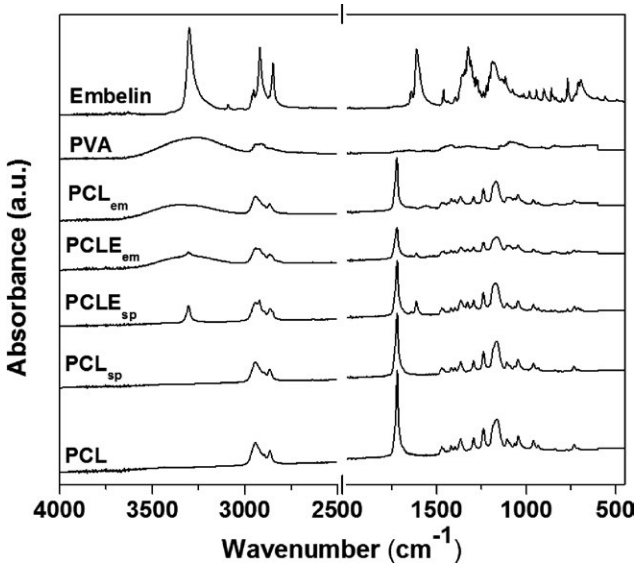

FIGURE 4 ATR-FTIR spectra of embelin, PCL, PVA and microparticles (PCL_{sp}, PCL_{em}, $PCLE_{em}$ and $PCLE_{sp}$). PCL, poly(ε-caprolactone); PVA, poly(vinyl alcohol); PCLE, poly(ε-caprolactone) microparticles containing embelin

in the particles.[44] Standard deviation of mean diameter increased with the incorporation of embelin, the value of standard deviation of $PCLE_{sp}$ being almost half of the value measured for $PCLE_{em}$.

Polydispersity index is an index of width or spread or variation within the particle size distribution. Thus, monodisperse samples have a lower PDI value, whereas higher values of PDI indicate a winder particle size distribution and the polydispersity nature of the sample. The usual range of PDI values is 0–0.05 (monodisperse standard), 0.05–0.08 (nearly monodisperse), 0.08–0.7 (middle range polydispersity), and higher than 0.7 (very polydisperse).[45] The PDI values showed that PCL_{em} and $PCLE_{em}$ microparticles have nearly monodisperse distribution (0.06) and middle range polydispersity (0.11) respectively. On the other hand, PCL_{sp} and $PCLE_{sp}$ particles have PDI values lower than 0.04, indicating a monodisperse standard distribution. Thus, EHDA allowed the preparation of microparticles with lower size dispersion.

It is known that optimal aerosol size is in the range of 1–5 μm for inhalation purposes.[42,46] Besides, PCL has been proposed for pulmonary delivery.[47] Then, $PCLE_{sp}$ microparticles with mean diameters in the aforementioned range result very attractive to administer embelin by inhalation therapy.

Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle, and its magnitude gives an indication of the potential long-term stability of particles in suspension. The surface charge depends on ionization of surface groups, pH, conductivity, and components concentration, among others. Particles with zeta potentials more positive than +30 mV or more negative than −30 mV are normally considered stable, since repulsive forces avoid particle aggregation. PCL particles exhibited low zeta potential values, and then there is no force to prevent the particles coming together leading to dispersion instability. However, the stability of embelin-loaded PCL particles is improved, as proved by zeta potential values more negative than −30 mV and SEM micrographs (Figures 2d and 3b). A possible contribution of anionic species derived from enolic and quinonic oxygens of the embelin structure could be the reason for observed decrease in zeta potential values of PCLE particles.

Surface composition of microparticles was studied by ATR-FTIR, and spectra are shown in Figure 4. Embelin showed characteristic peaks at 3,300 cm^{-1} (νO–H; 2,918 cm^{-1} and 2,848 cm^{-1} (ν_aC–H and ν_sC–H in CH_2), and 1,612 cm^{-1} (νC=C), 1,462 cm^{-1} (δC–H in $–CH_2$), 1,375 cm^{-1} (δC–H in $–CH_3$), 1,219 cm^{-1}, (νC=O). PCL spectra exhibited the typical signals of polyesters at 2,943 and 2,864 (ν_aC–H and ν_sC–H in CH_2), 1,720 cm^{-1} (νC=O in ester), 1,398 cm^{-1} and 1,368 cm^{-1} (δC–H in CH_2), 1,246 cm^{-1} and 1,194 cm^{-1} (νC–O–C in ester), and 1,108 cm^{-1} (νC–O–C in ether). In the case of PVA, a broad peak is observed in the region of 3,620–3,000 cm^{-1} associated to the O–H stretching corresponding to inter and intramolecular hydrogen bonding, and peaks in the region 2,900–2,800 cm^{-1} associated to C–H stretching of alkyl groups.

The observation of these signals in the spectra of PCL_{em} and $PCLE_{em}$ microparticles indicates the presence of the emulsifier PVA at the surface of microparticles obtained by emulsion solvent evaporation, even after extensive washing. Sahoo et al.[48] reported that PVA forms an interconnected network with the polymer at the interface and affects the physical properties and cellular uptake of particles. The amount of residual PVA depends on concentration of PVA and the type of organic solvent used in the emulsion, and could be up to 13% w/w of the nanoparticle. The residual PVA, in turn, influenced different pharmaceutical properties such as particle size, zeta potential, surface hydrophobicity, drug loading,

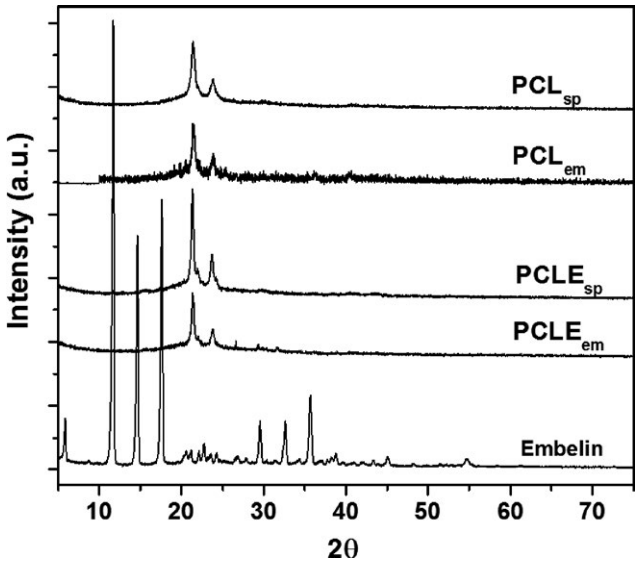

FIGURE 5 XRD patterns of embelin and microparticles (PCL$_{sp}$, PCL$_{em}$, PCLE$_{sp}$ and PCLE$_{em}$). XRD, X-ray diffraction; PCL, poly(ε-caprolactone); PCLE, poly(ε-caprolactone) microparticles containing embelin

and slightly influence the in vitro release of the encapsulated drug. The PVA macromolecules could swell forming a hydrogel barrier to the diffusional release of drug. Thus, the percentage of residual PVA is an important parameter and it must to be taken into account to analyze the pharmaceutical properties of drug-loaded microparticles.

ATR-FTIR spectra of PCLE$_{em}$ and PCLE$_{sp}$ microparticles showed peaks associated to the presence of embelin at the surface (3,305 and 1,614 cm^{-1}). Therefore, embelin could be responsible for the observed zeta potential value with respect to the ones displayed by plain particles (PCL$_{em}$ and PCL$_{sp}$). In the case of the PCLE$_{sp}$ microparticles, the surface enrichment with embelin could be attributed to the fast solvent evaporation that occurred in EHDA technique, and the segregation of embelin because of its low solubility in PCL.

Figure 5 shows XRD diffractograms of embelin and microparticles. Embelin exhibited several intense peaks at $2\theta = 5.81°$, 11.70°, 14.63°, 17.59°, 29.53°, 32.63°, and 35.69°. However, these peaks were not observed in the XRD patterns from PCLE microparticles. This indicates that the drug would be molecularly dispersed within the polymer or distributed in an amorphous state or crystalline with very small size. The patterns from microparticles correspond to characteristic peaks showed by PCL ($2\theta = 21.40°$ and 23.75°).

3.2 | Thermal properties

Thermal properties are summarized in Table 2. Embelin crystallizes in the form of golden yellow needles, displaying three melting endotherms. As could be seen in DSC curves, embelin melting peaks are suppressed in PCLE$_{sp}$ and PCLE$_{em}$ microparticles, indicating a very good dispersion of embelin in the PCL matrix. This is in agreement with the absent of embelin peaks in XRD patterns, as mentioned before.

On the other hand, PCL crystallinity in both types of microparticles decreased with respect to PCL pellets. This variation is associated to the processing techniques used to prepare the microparticles, which affect the crystallization process in many ways. In the case of EHDA, the solvent evaporation occurred between the nozzle and the collector in a short period of time, and even under these conditions PCL showed capacity to crystallize. No other relevant change in the physical properties of PCL after EHDA process was observed. Moreover, degradation or chemical changes were not observed, as evidenced by FTIR.

3.3 | Drug loading capacity and encapsulation efficiency

High loading capacities (LC) are desirable for an increased availability of drugs with minimal used of carrier. However, an increase in loading capacity led to a loss of sphericity during solvent evaporation and particle shrinkage, leading to a corrugated morphology which affects drug release profiles. These morphological changes could be associated to the migration of embelin toward the particle surface and the fast chloroform evaporation. Actual loading capacities in EHDA have shown to be slightly decreased (typically 20%) compared to theoretical loadings.[48] In this work, embelin LC of PCLE$_{em}$ was 1.05%, whereas the value for PCLE$_{sp}$ reached 6.1% (Table 1).

TABLE 2 Thermal properties of embelin, PCL, and microparticles (PCLE$_{em}$ and PCLE$_{sp}$): melting enthalpy of PCL (ΔH_m); melting enthalpy of embelin (ΔH_{mE}); melting temperature of PCL (T_m); melting temperature of embelin (T_{mE}); crystallinity degree of PCL (X_{cPCL})

Sample	T_m (°C)	ΔH_{mPCL} (J/g)	X_{cPCL} (%)	ΔH_{mE} (J/g)
PCL pellet	65.6	87.86	59.3	—
PCL$_{em}$	60.5	57.93	43.0	—
PCL$_{sp}$	59.0	70.69	47.7	—
Embelin	81.7; 87.1	—	—	63.45
	145.8			129.68
PCLE$_{em}$	59.0	58.80	43.7	—
PCLE$_{sp}$	58.6	66.23	47.6	—

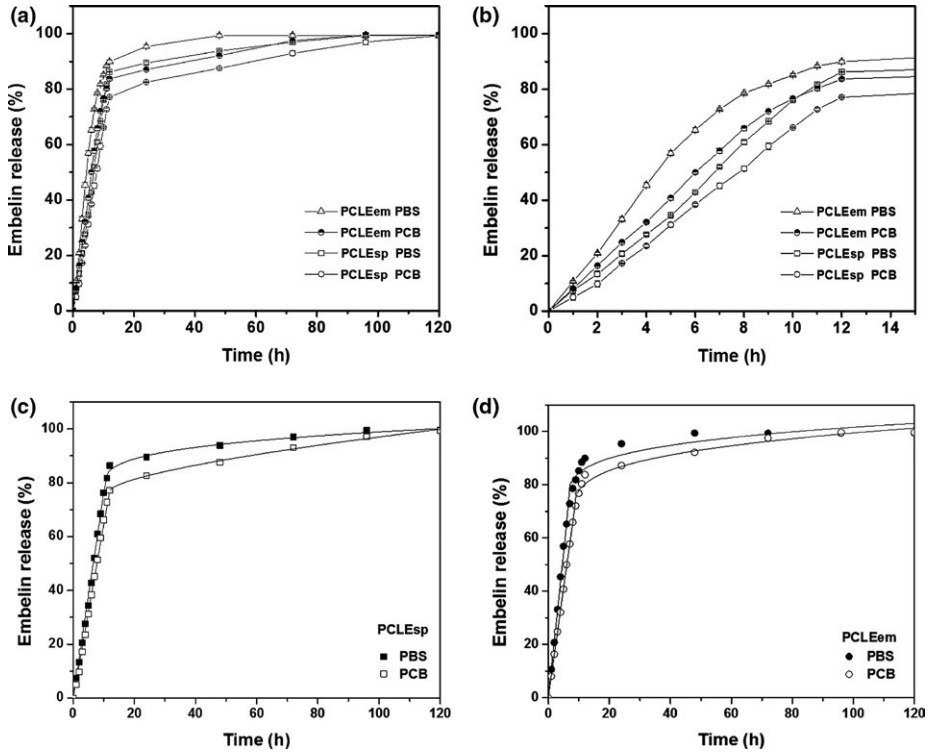

FIGURE 6 Embelin release profiles: (a) $PCLE_{em}$ and $PCLE_{sp}$ microparticles in PBS and PCB; (b) $PCLE_{em}$ and $PCLE_{sp}$ microparticles in PBS and PCB during the first 15 hr; (c) Data fitting for $PCLE_{sp}$ microparticles in PBS (pH = 7.4) and PCB (pH = 5.0); (d) Data fitting for $PCLE_{em}$ microparticles in PBS (pH = 7.4) and PCB (pH = 5.0). PCB, phosphate-citrate buffer; PBS, phosphate buffered saline; PCLE, poly(ε-caprolactone) microparticles containing embelin

On the other hand, encapsulation efficiency (EE) depends on the combination of drug/solvent/polymer of each particular system, where the hydrophilic/hydrophobic characteristics of each component play an important role. In the case of emulsion solvent evaporation technique, $PCLE_{em}$ microparticles had an EE of 10.65%, indicating that this process allowed the encapsulation of embelin of only 1% w/w. The presence of both aqueous and organic phases in the emulsion procedure, may lead to preferential diffusion of drug to one phase or the other according to their hydrophilicity, and thus reducing the EE.[49] Moreover, the PVA removal during washing procedures additionally reduces the embelin content due to solubility loss. Higher amounts of embelin led to surface enrichment due to embelin rejection. Our experimental results indicated that a concentration of embelin in PCL of 10% w/w can be considered as the saturated solution.

Electrohydrodynamic atomization is a technique that yields high EE, reaching in some cases a value close to 100%. PCLEsp microparticles exhibited an EE of 64.75%, corresponding to a concentration of embelin of 6.5% w/w. The partition coefficient considers the equilibrium solubility of drug/polymer against drug/solvent. As embelin is highly soluble in chloroform, a preferential diffusion into the solvent could reduce the EE. The presence of embelin at the nozzle tip after the electrospraying process explains the observed behavior. Toman et al.[50] compared drug loading efficiencies of alkylglyceryl-modified dextran-graft-poly(lactic acid) nanoparticles prepared by solvent displacement and electrospraying. They also found that electrospraying provided a more efficient loading method compared to solvent displacement for doxorubicin-loaded nanoparticles.

Finally, if Equation (4) were used to determine encapsulation efficiency, $PCLE_{sp}$ microparticles would have an EF of approximately 92%. It is evident that this expression overestimates the efficiency because it considers that polymer mass remained constant before and after processing, without taking into account losses produced during particle preparation. Using that equation, efficiencies for PCL microparticles with encapsulated paclitaxel[39] and β-oestradiol-loaded PCL microparticles[43], were reported as 80% and 89% respectively.

3.4 | Study of the release behavior from PCLE microparticles

It is known, that in the early stages, the extracellular pH do not change dramatically, and the embelin release favors the tumor targeting. In the case of advanced stages, the extracellular pH can decrease up to 5. This decrease in pH favors the growth of tumor cells and the ability of tumor cells to form lung metastases.[51] On the other hand, the intracellular pH on tumor cells is maintained at neutral range by means of powerful pH control mechanisms. The low extracellular pH and

the significant gradients between extracellular and intracellular pH affect markedly the response of tumors to various treatments such as chemotherapy, radiotherapy, and hyperthermia.[51] In this way, the release studies were carried out at pH = 7.4 and pH = 5.0 which correspond to two extreme conditions. Figure 6a, b show the in vitro release profiles from $PCLE_{em}$ and $PCLE_{sp}$ microparticles in PBS (pH = 7.4) and PCB (pH = 5.0) at 37°C. Embelin was released following a profile with two stages. First, a high release rate is observed in the initial stage, normally known as burst effect. One suggested explanation for the burst effect in monolithic systems is that some drug becomes trapped on the surface of the polymer matrix during manufacturing process, and is released immediately upon activation in a release medium.[52] Migration of drugs solvent evaporation may result in a heterogeneous distribution of drug in the polymer matrix that leads to this effect. In the second stage, a diffusion-controlled mechanism was observed.

In the case of $PCLE_{em}$ particles, the release profiles in PBS showed a burst release during the first 6 hr, where a 65% of the drug is released, and the rest at 48 hr. In PCB, the release was slower; burst effect was prolonged until 8 hr to release the same amount that in PBS, and the total drug was released at 96 hr.

The analysis of release behavior of $PCLE_{sp}$ indicates that burst effect was maintained during the first 10 hr (76%), reaching the complete release at 120 hr (5 days). As in the other system, the first stage in PCB was prolonged to 12 hr, also reaching approximately the same amount (77%). The process was also completed in 120 hr. These results indicate that the predominant mechanism of release in the first hours was drug dissolution, and was dependent on pH and drug solubility in the medium. In the second stage, embelin solubility had no effect on the observed profiles.

Embelin is an alkyl substituted hydroxybenzoquinone compound that contains two enolic hydroxyl groups attached to a hydrocarbon ring at 2 and 5 positions. These hydroxyls are very weak acids. The solubility in water of embelin is 130 μg/ml.[21] According to the solubility criteria given by the United States Pharmacopeia (USP) and the National Formulary (NF), embelin is a very slightly water-soluble drug (USP, 2010).

In alkaline media, deprotonated anions of enols (enolates) are formed. The resulting structure, known as embilinate anion, acts as a bidentate ligand and has demonstrated to be involved in complexation with divalent metal ions through its enolic and quinonic oxygens atoms located at positions 2 and 1.[53] Moreover, the embelinate anion can be stabilized by the resonance, since the anionic charge can be delocalized over the carbonyl located at position 4. This delocalization increases stabilization of the anion, and then increases the acidity of hydroxyl protons and water solubility. For this reason, in PCB (pH = 5) the solubility is expected to be lower than in PBS (pH = 7.4), and a different release behavior is observed. Accordingly, the slight difference observed in the release behavior could be associated to the solubility, and pH change does not affect the overall behavior.

The processing technique used to prepare PCLE microparticles also showed an influence in the observed release behavior. It is known that loading affect burst release with a higher loading leading to a higher burst release. Accordingly, $PCLE_{sp}$ presented the higher burst release followed by a slower release phase. The higher facility to release embelin from $PCLE_{em}$ could be ascribed to the residual PVA found in these particles. Sahoo et al.[48] reported that PVA macromolecules could swell forming a hydrogel barrier to the diffusional release of proteins. However, in the second stage, embelin was released from $PCLE_{em}$ more easily than in $PCLE_{sp}$, indicating that the residual PVA promoted water introduction into the particle and embelin dissolution. In fact, complete released from PCLEem in PBS occurred within 48 hr whereas for PCLEsp the process was carried out during 96 hr.

3.5 | Kinetic analysis of in vitro release profiles

The results of the kinetic analysis for $PCLE_{em}$ and $PCLE_{sp}$ at different pH are presented in Figure 6b,c respectively. The parameters derived from fitting models of controlled release mechanisms to experimental data and coefficient of determination (R^2) are listed in Table 3.

The time in which the linear behavior finishes and begins the diffusional stage (t_b) is also showed in Table 3. Burst release may be the optimal mechanism of delivery in several

TABLE 3 Model parameters corresponding to $PCLE_{em}$ and $PCLE_{sp}$ in PBS (pH = 7.4) and PCB (pH = 5), as determined by curve fitting

Sample and buffer		t^a (hr)	Higuchi model		Korsmeyer-Peppas model		
			K_H ($hr^{-1/2}$) (*SD*)	R^2	K_{KP} (hr^{-n}) (*SD*)	*n* (*SD*)	R^2
PBS	$PCLE_{sp}$	10	2.559 (0.192)	0.842	6.867 (0632)	0.265 (0.022)	0.984
	$PCLE_{em}$	6	4.339 (0.515)	0.299	14.432 (1.555)	0.203 (0.029)	0.901
PCB	$PCLE_{sp}$	12	2.048 (0.076)	0.976	1.027 (0.162)	0.660 (0.036)	0.995
	$PCLE_{em}$	8	3.814 (0.312)	0.771	9.936 (0.932)	0.269 (0.023)	0.970

[a]Time to complete the initial burst.

instances, but it is difficult to control and predict. Many drugs need to be administered at varying rates, and for some treatments, such as the initial phase of wound treatment, a burst release provides immediate relief followed by prolonged release to promote gradual healing.

Mathematical models (Higuchi and Korsmeyer-Peppas) commonly used to describe the diffusion release profile were tested through non-linear least-square curve fitting (Equations 5 and 6). Korsmeyer-Peppas model showed the best-fit curve for $PCLE_{em}$ and $PCLE_{sp}$ microparticles in the investigated mediums. The constant K_{KP} and n are characteristics of the drug-polymer system. The diffusional exponent is dependent on the geometry of the device and the physical mechanisms of release. Embelin release from $PCLE_{sp}$ presented the highest goodness of fit ($R^2 = .984$ and.995 in PBS and PCB respectively). For classical Fickian diffusion in spherical devices, the diffusional exponent is $n = 0.43$. The best-fit curves in PBS indicated a quasi-Fickian diffusion mechanism ($n < 0.43$) for both types of particles. On the other hand, an anomalous transport or non-Fickian diffusion ($0.43 < n < 0.85$) was observed in PCB for $PCLE_{sp}$, whereas $PCLE_{em}$ displayed a quasi-Fickian diffusion mechanism.

4 | CONCLUSIONS

For the first time, PCL microparticles containing embelin were successfully prepared by electrohydrodynamic atomization and emulsion solvent evaporation techniques. $PCLE_{sp}$ microparticles presented the best physical, morphological, and drug-loading features as compared with $PCLE_{em}$. EHDA-processed microparticles exhibited a sixfold encapsulation efficiency with respect to $PCLE_{em}$, considering than both types of microparticles present sizes within the optimum range for inhalation therapies. In vitro embelin release from PCLE displayed profiles with a high initial release, followed by a slow diffusional phase. In summary, electrosprayed PCLE microparticles developed in this work presented characteristics, size, zeta potential, and release profiles that could be very attractive for targeted embelin administration, such as inhalation therapy for lung cancer. Finally, it can be concluded that EHDA is the most suitable technique for preparation of embelin-loaded microparticles.

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