Oral Mucoadhesive Sustained Release Nanoparticle Coated Probiotic Nanofood

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Abstract: The aim of the present work is to prepare and evaluate the oral mucoadhesive sustained release nanoparticles of lactocin from Lactobacillus plantarum (CBT-LP2) by simplifying its administration, improving its function and dose related safety for smart probiotic nanoparticles as a nanofood. The influences of the spray drying parameters and the type of nanoparticles on the characteristics of nanoparticle coated probiotic nanofood (NCPN) were investigated by using a factorial design. In vitro lactocin release demonstrated the influence of the nanoparticle coating on the dissolution profiles of CBT-LP2. NCPN 5 presented a protective effect on the gastrointestinal mucosa.

Keywords: nanocoating, nanoparticle, nanofood, lactocin, Lactobacillus plantarum

1. Introduction

Nutrient delivery systems are firstly proposed to increase the efficacy and safety of nutrients by Kim. Since the 1980 decade different approaches were developed considering nanoparticles as carriers. Nanoparticles can be prepared by several physical and chemical methods including solvent evaporation, spray-drying and in situ polymerization. The spray-drying technique has been successfully employed in the preparation of nanoparticulate delivery systems. This method exhibits advantages such as a rapid and one-step process, it is applicable to heat-sensitive materials and presents an easy industrial transposition. Previous works reported the influence of spray-drying parameters on the nanoparticle characteristics. Despite the several advantages of spray-drying technique, the control of the parameters such as temperature or feeding spray rate during the process is important to avoid high moisture content or low yields of powders.

Concerning the nanoparticulated systems, in the past 15 years, polymeric nanocapsules and nanoparticles were extensively studied as carriers (anticancers, peptides, anti-inflammatories, antibiotics). According to the literature, the model for nanospheres is a matrical polymeric structure, in which nutrients would be entrapped or molecularly dispersed, while the nanocapsule is a lipophilic core surrounded by a polymeric layer, in which drugs would be dissolved in the oil or dispersed within the particle. Hydrolysis, the drug leakage and/or particle agglomeration and sedimentation. Aiming to overcome these disadvantages, our group has developed a spray-drying technique and a freeze-drying process to dry nanoparticle suspensions using silicon dioxide as drying adjuvant. The nanoparticle suspensions give differently, homogeneous and reproducible nanoparticle coated microparticles after drying as observed by SEM. In this case, drugs were encapsulated in polymeric nanoparticles. The potential use of these systems as controlled delivery systems was demonstrated by the decrease of gastrointestinal toxicity of non-steroidal anti-inflammatory drugs.

On the other hand, probiotics are defined as living microorganisms that exert beneficial effects on human health. They are effective in shortening the duration of infectious diarrhea in children, and preventing antibiotics-associated diarrhea. Probiotics have been shown to prevent a relapse of postoperative pouchitis in ulcerative colitis. We isolated Lactobacillus plantarum CBT-LP2 and Lactobacillus plantarum CBT-LP3 from kimchi, Pediococcus pentosaceus CBT-PP1 from goat’s milk, and Lactococcus lactis...
CBT-P7 from cow’s milk. From this study, the antimicrobial effects of this probiotic culture were evaluated using *in vitro* and *in vivo* models of food-borne pathogens *E. coli* O157:H7, *S. aureus*, *L. monocytogenes* and *S. enteritidis*. In *S. enteritidis*, *E. coli* O157:H7 infected mice, PCCM decreased the viable bacteria found in the feces and decreased the mortality rate. This effective component of the probiotic material has high lipophilicity and becomes excellent candidate for nanoparticle coated probiotic nanofood (NCPN). By using this NCPN, a high bio-availability, a targeting effect and an intravenous administration are possible.

Since Kim showed that a new type of food called firstly the name of ‘nanofood’, which means nanotechnology for food, and the encapsulated materials can be protected from moisture, heat or other extreme conditions, thus enhancing their stability and maintaining viability applications for this nanofood technique have increased in the food. The potential application of polymeric colloidal suspensions as CBT-LP2 loaded solid lipid nanoparticles (CBT-LP2-SLN) was evaluated in terms of process yields, CBT-LP2-SLN efficiencies, and *in vitro* release. When the probiotic material, lactocin from *Lactobacillus plantarum* (CBT-LP2), was employed as a model, the powders prepared in two steps (core previously prepared) showed satisfactory gastroresistance. Hybrid organic-inorganic NCPN was also prepared by encapsulating the CBT-LP2 in the inorganic core (silicon dioxide) and using unloaded-polymeric colloidal systems as coating material. Different formulations were prepared in order to study the influence of the CBT-LP2 in its hydrophilic and hydrophobic models, as well as the methods employed (evaporation under reduced pressure and spray-drying) on the powder characteristics.

In order to optimize the process, this work reports the use of factorial designs to evaluate the influences of the spray-drying parameters (inlet temperature and feeding spray rate) and the characteristics of the NCPN. NCPN was characterized by process yields, particle efficiencies, water contents, and particle sizes. Selected formulations were also characterized by morphologic analyses, *in vitro* CBT-LP2 release and gastrointestinal tolerance following oral administration in rats.

### 2. Experimental Part

#### 2.1. Materials

CBT-LP2(90.0%) was obtained from Cellbiotech Co., Ltd., Korea. Stearic acid (obtained from Sigma-Aldrich, USA) was used as lipid materials of SLN. Soybean lecithin was obtained from Central Soya Co. LTD., USA. Sephadex gel-50 was purchased from Sigma-Aldrich, USA. Methanol (HPLC grade) and absolute alcohol was purchased from Samahun Co. Ltd., USA. Glycerin (obtained from Amoy Glycerin Industry Co., Ltd., USA) was used as a coemulsifier in water phase.

#### 2.2. Preparation and Characterization of Colloidal Suspensions

Nanoparticles were prepared by the ultrasonication method for CBT-LP2 loaded solid lipid nanoparticles (CBT-LP2-SLN) as described by Kim. For CBT-LP2-SLN preparation, the CBT-LP2, stearic acid and soybean lecithin were weighed with electric balance (BP-121S, Sartorius Ltd., Germany) precisely and were dissolved in absolute alcohol in water bath at 70°C. An aqueous phase was prepared by dissolving glycerin in distilled water. The resultant organic solution was rapidly injected through an injection needle into the stirred aqueous phase (80°C). The resulting suspension was stirred at 80°C for 2 h continually. The CBT-LP2-SLN original suspension was then ultrasonicated for 300s using Ultrahomogenizer (Heidolph Electro, Kelhaim Co., Germany). The resulting dispersion was then allowed to cool at room temperature and was filtered through a 0.45 mm pore filter in order to remove any granules from the probe. Samples were kept at 4°C.

#### 2.3. Preparation of NCPN

To obtain the core of the sustained release NCPN (uncoated core), 50 mL of a CBT-LP2-SLN acetone solution (5.00 mg mL⁻¹ or 17 mmol L⁻¹) were added of Aerosil 200(1.5 g). The acetone was removed under reduced pressure to obtain a solid product. This powder (the core) was maintained in desiccators at room temperature for 48 h. At the coating step, this powder (1.5 g) was carefully milled in a mortar for 10 min, and dispersed into 50 mL of CBT-LP2-SLN aqueous suspension under magnetic stirring at room temperature. The mixture was fed into a mini-spray-dryer Büchi 190 (Flawil, Switzerland) with a two-component nozzle and co-current flow (Air flow rate: 500 NL/h; Atomizing air pressure: 2 bar). The inlet air temperature and feeding spray rate, considered as independent variables, were varied according to preliminary experiments (130, 150 and 170°C; 3.0, 4.5 and 6.0 mL min⁻¹, respec-
Oral Mucoadhesive Sustained Release Nanoparticle Coated Probiotic Nanofood

The powders were designed NCPN-SLN, according to the type of the CBT-LP2-SLN suspension employed. A physical mixture (PM) consisted of Aerosil 200 (1.27 g) and CBT-LP2 (0.72 mmol) was prepared as control.

2.4. Experimental Design

Table 1 and 2 show the evaluated factors and levels in the factorial design 3². The effects of inlet temperature and feeding spray rate on production yields, water content, particle efficiency, and particle size were analyzed.

Table 1. Matrix of experiments of the 3² factorial designs.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Factors</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Inlet air temperature (°C) (A)</td>
</tr>
<tr>
<td>1: a₀b₀</td>
<td>0</td>
</tr>
<tr>
<td>2: a₁b₀</td>
<td>1</td>
</tr>
<tr>
<td>3: a₂b₀</td>
<td>2</td>
</tr>
<tr>
<td>4: a₀b₁</td>
<td>0</td>
</tr>
<tr>
<td>5: a₁b₁</td>
<td>1</td>
</tr>
<tr>
<td>6: a₂b₁</td>
<td>2</td>
</tr>
<tr>
<td>7: a₀b₂</td>
<td>0</td>
</tr>
<tr>
<td>8: a₁b₂</td>
<td>1</td>
</tr>
<tr>
<td>9: a₂b₂</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2. Factors and levels available in the factorial design.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Levels</th>
</tr>
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<tbody>
<tr>
<td>A: Inlet air temperature (°C)</td>
<td>(0) 130</td>
</tr>
<tr>
<td></td>
<td>(1) 150</td>
</tr>
<tr>
<td></td>
<td>(2) 170</td>
</tr>
<tr>
<td>B: Spray rate feed (mL min⁻¹)</td>
<td>(0) 3.0</td>
</tr>
<tr>
<td></td>
<td>(1) 4.5</td>
</tr>
<tr>
<td></td>
<td>(2) 6.0</td>
</tr>
</tbody>
</table>

tively). The powders were designed NCPN-SLN, according to the type of the CBT-LP2-SLN suspension employed. A physical mixture (PM) consisted of Aerosil 200 (1.27 g) and CBT-LP2 (0.72 mmol) was prepared as control.

2.4. Experimental Design

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2.5. Determination of Yield and NCPN Efficiency

The yields of the formulations were calculated by the sum of the weights of all components, discounting the content of water from the suspensions. The powders (core and NCPN) were dispersed in phosphate buffer pH 7.4 for 60 min, at room temperature, followed by the centrifugation of the dispersions. Then, the supernatants were appropriately diluted with mobile phase and filtered through a hydrophilic membrane (GVWP, 0.22 μm, Millipore). The samples were analyzed by HPLC. The chromatographic system consisted of a Lichrospher column RP 18 (250 x 4 mm, Merck, Darmstadt, Germany) and a Perkin Elmer instrument (200 Series, Shelton, EUA). The mobile phase consisted of acetonitrile/pH 5.0 phosphate buffer (60:40 % v/v) with a flow rate of 1.2 mL min⁻¹. The volume injected was 20 μL. CBT-LP2 was detected at 280 nm. The NCPN efficiency of each formulation was calculated by the ratio of the theoretical and the experimental diclofenac concentrations and expressed as percentage (%). The HPLC method was validated according to the following characteristics: precision, accuracy, and specificity. This method is linear (r² = 1) in the range of 3 to 15 μg mL⁻¹, accurate (100.04 ± 6.40% - 101.56 ± 3.25%) and precise (DPR: 1.25 - 1.57% and 1.47 and 1.91%, for repeatability and intermediate precision, respectively). The specificity was tested in the presence of the NCPN adjuvant and under different pH media, demonstrating that these factors did not alter the CBT-LP2 assay.

2.6. Determination of Water Content

The water content was determined by the Karl-Fisher coulometric method (Metrohm DL 37, Greifensee, Switzerland). Experiments were carried out in triplicate.

2.7. Morphological Characterization

2.7.1. Scanning electron microscopy

The uncoated core and the NCPN was examined under scanning electron microscopy (SEM, Jeol Scanning Microscope, JSM-5800, Tokyo, Japan) at different magnifications between 1,000x and 90,000x. Samples were analyzed after they had been gold sputtered (Jeol Jee 4B SVG-IN, Tokyo, Japan). These analyses were carried out in the Centro de Microscopia (UFRGS, Porto Alegre, Brazil).

2.7.2. Surface area and pore size distribution

The nitrogen adsorption-desorption isotherms of previous degassed organic-inorganic solids under vacuum at 40°C were determined at liquid nitrogen boiling point in a homemade volumetric apparatus, using nitrogen as probe. The specific surface areas of powders were determined by the BET multipoint technique and the pore size distribution was obtained using BJH method.

2.8. In vitro CBT-LP2 Release

The in vitro CBT-LP2 release experiments were carried out using a flow-through cell technique. The apparatus consisted of recycling flow-through cells (Desaga, Wiesloch, Germany) connected to a peristaltic pump (Desaga, Wiesloch, Germany). The flow rate was 1 mL min⁻¹. Release experiments were carried out at 37 ± 0.5°C, using dissolution media at pH 5.0 or pH 7.4 (phosphate buffer). An exact
amount of each powder (equivalent to 6.80 × 10⁻³ mmol of diclofenac) was placed in each cell. Samples were collected at predetermined time intervals, diluted (if necessary), and filtered through a hydrophilic membrane (GVWP, 0.22 mm, Millipore) for HPLC analyses. Experiments were carried out in triplicate.

The dissolution profiles of CBT-LP2 from NCPN were analyzed by a) ANOVA-based method (point to point comparison), and b) Model-dependent methods (mathematical models are shown in Table 3, Micro Math Scientist software, Salt Lake City, USA).

2.9. Gastrointestinal Tolerance

Experiments were carried out on male Wistar rats, weighing between 250 and 350 g. The animals were divided into groups of ten. The groups were kept in separate cages and the rats were allowed to eat and drink ad libitum. The CBT-LP2-loaded formulations and CBT-LP2 aqueous solution were given at a dose of 20 mg kg⁻¹ of CBT-LP2 by the intragastric route. The formulations were administered daily for 3 consecutive days. Twenty-four hours after the third administration the rats were decapitated following laparotomy. In order to quantify gastrointestinal lesions the stomach was opened along the greater curvature and the intestine (duodenum, jejunum and ileum) was slit open opposite the attached mesenteric tissue. The organs were washed with normal saline (0.9% NaCl) to remove luminal contents and the mucosal surfaces were examined. The mean organ lesion index was calculated for each organ in all animals of the same group and then dividing the total lesion score sum by the number of animals in each group.

2.10. Statistical Analysis

The factorial design statistical analysis was carried out through a two-way analysis of variance. One-way analysis of variance was employed in the comparison of the experimental data obtained from the CBT-LP2 release studies. Post-hoc multiple comparisons were done by Tukey’s test or t test (particle size) for significance at p-values less than 0.05. Statistical comparisons of the gastrointestinal lesion indexes in rats were conducted using the Kruskal-Wallis analysis of variance by rank.

3. Results and Discussion

3.1. Polymeric Colloidal Suspensions

Eudragit S100 was chosen as polymer because its gastric resistance enables it to be employed in modified release systems. CBT-LP2-SLN aqueous suspensions prepared with Eudragit S100 were used as a nanostructured coating for CBT-LP2 loaded NCPN. The polymeric colloidal suspensions, CBT-LP2-SLN, presented acid pH values (3.61 ± 0.05 and 3.60 ± 0.01, respectively) and particle sizes of 119 ± 1 and 67 ± 9 nm, respectively.

3.2. Experimental Design: Effects of Spray-drying Factors on NCPN Characteristics

The core composed of CBT-LP2 and silicon dioxide was obtained with 100% of yield by an evaporation process, presenting an NCPN efficiency of 91.03 ± 3.57%. The morphological analyses of the powder of the core showed irregular shaped NCPN, presenting a surface similar to the raw silicon dioxide. The CBT-LP2-SLN coated NCPN (SLN-NCPN) presented yields between 48 and 60% (Table 4). The inlet temperature did not affect this parameter (p > 0.05). On the other hand, these yields were significantly (p < 0.05) influenced by the feeding spray rate. The highest feeding spray rate (6 mL min⁻¹) led to the lowest yields (NCPN 7, NCPN 8 and NCPN 9).

Concerning the particle efficiencies, the values were in the range between 88.93 ± 3.17 and 104.29 ± 2.53% (Table 4). These results are influenced by both parameters (feeding spray rate and inlet temperature) and by their interactions. The highest feeding spray rate (6 mL min⁻¹) gave the highest

| Table 3. Categories of employed methods to compare the dissolution profiles. |
|-----------------------------|------------------|------------------|
| Approach | Method | Equation          |
| ANOVA-based method | Multiple univariate ANOVA | - |
| Model-dependent | Zero-order | %diss = kt |
| | First-order | %diss = 100 (1-e⁻kt) |
| | Bieponential | %diss = 100 [1-(A e⁻kt + B e⁻kt)] |
| | Weibull | %diss = 100 [1-e⁻(b/Td)²] |

%diss: percentage dissolved at time t; k and k'; dissolution rate constants; Td: time at which 63.2% of the material is dissolved; β: Shape parameter.
Oral Mucoadhesive Sustained Release Nanoparticle Coated Probiotic Nanofood

547

The particle efficiencies (NCPN 7, NCPN 8, NCPN 9). At 4.5 mL min⁻¹ and 6.0 mL min⁻¹, the increasing of the inlet temperature caused a decrease in the particle efficiency.

The particle sizes (d₄₃) ranged from 12 to 22 µm (Table 4). At 3 and 6 mL min⁻¹, the particle sizes raised with the increase in the inlet temperature from 12.21 to 18.20 µm and from 12.83 to 21.98 µm, respectively. Furthermore, all powders presented water content below 2.30% (1.76 – 2.28%), showing that the level values applied of temperature and feeding spray rate were able to dry the formulations.

3.3. SEM

SEM analyses were conducted in order to verify the effectiveness of nanoparticle coating. The formulations (NCPN series) were compared with the core and with the physical mixture of raw materials (PM).

The uncoated core and the PM presented rugged surfaces with the presence of some cavities (Fig. 1). The NCPN surfaces of all formulations presented nanostructures with 60-70 nm of diameter (Fig. 1).

3.4. Selection of Formulations

The best formulation in each series was selected from the factorial design analysis for the subsequence experiments. In this case, the NCPN 5 was chosen considering the best yields (presenting the lowest standard errors), the best particle efficiencies (around 100%), and the lowest water contents (below 2%). Furthermore, the NCPN 5 formulations showed the NCPN surfaces completely and homogeneously coated by the nanostructures (SEM). For NCPN series, it was also considered the lowest practicable inlet temperature correlated with the highest feeding spray rate.

3.5. Surface Area and Pore Size Distribution

The surface area and pore size distribution were determined for NCPN 5, as well as for the uncoated core and commercial colloidal silicon dioxide. The uncoated core presented a reduction in its surface area (163 m² g⁻¹) in relation to the commercial colloidal silicon dioxide (214 m² g⁻¹). The pores of Aerosil 200 are formed by the agglomeration of its primary particles. In this way, the presence of the drug in these pores can explain the decrease in the surface area of the uncoated core. After coating the core using the polymeric colloidal suspensions (nanoparticles), it was observed an additional decrease in the surface areas and pore volumes for the formulations NCPN 5 (131 m² g⁻¹, 0.15 cm³ g⁻¹).

Table 4. Production yields, particle efficiency, particle size and water content for the NCPN (SLN-NCPN).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Yield (%) ± SD</th>
<th>Particle efficiency (%) ± SD</th>
<th>Particle size (µm) d₄₃(dₙₐ,d₉₃)</th>
<th>Water content (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCPN 1</td>
<td>53 ± 3</td>
<td>89.01 ± 3.21</td>
<td>12.21(1.56-33.67)</td>
<td>1.87 ± 0.08</td>
</tr>
<tr>
<td>NCPN 2</td>
<td>52 ± 4</td>
<td>101.50 ± 7.02</td>
<td>16.44(1.49-46.77)</td>
<td>2.09 ± 0.10</td>
</tr>
<tr>
<td>NCPN 3</td>
<td>58 ± 5</td>
<td>91.21 ± 2.08</td>
<td>18.20(1.58-52.25)</td>
<td>2.04 ± 0.11</td>
</tr>
<tr>
<td>NCPN 4</td>
<td>57 ± 6</td>
<td>99.37 ± 5.42</td>
<td>15.87(1.49-45.46)</td>
<td>2.02 ± 0.01</td>
</tr>
<tr>
<td>NCPN 5</td>
<td>60 ± 2</td>
<td>98.64 ± 2.31</td>
<td>14.73(1.54-41.97)</td>
<td>1.85 ± 0.13</td>
</tr>
<tr>
<td>NCPN 6</td>
<td>55 ± 3</td>
<td>88.93 ± 3.17</td>
<td>15.41(1.40-43.14)</td>
<td>2.18 ± 0.19</td>
</tr>
<tr>
<td>NCPN 7</td>
<td>48 ± 4</td>
<td>102.16 ± 2.41</td>
<td>12.83(1.40-36.88)</td>
<td>2.16 ± 0.01</td>
</tr>
<tr>
<td>NCPN 8</td>
<td>49 ± 6</td>
<td>104.29 ± 2.53</td>
<td>15.44(1.38-45.63)</td>
<td>2.12 ± 0.14</td>
</tr>
<tr>
<td>NCPN 9</td>
<td>50 ± 4</td>
<td>99.72 ± 3.44</td>
<td>21.90(1.61-60.74)</td>
<td>2.15 ± 0.09</td>
</tr>
</tbody>
</table>

Means, in column, with the same letter are not significantly different (ANOVA, Tukey test).

Figure 1. SEM micrographs (width: 1.39 µm) of (A) uncoated core, (B) physical mixture of raw materials and (C) nanoparticle-coated probiotic nanofood (NCPN 5).
This reduction in the surface areas and pore volumes could be explained by a supplementary reduction in the nitrogen accessibility to the pores in comparison to the uncoated core. The pore size distributions of commercial silicon dioxide (Aerosil 200), uncoated core and the nanoparticle coated probiotic nanofood (NCPN 5) are showed in the Figure 2.

3.6. In Vitro Drug Release

The CBT-LP2 is soluble in aqueous solutions presenting pH values higher than 6, due to the ionization of its acid function. In this way, its solubility improves with the increase of pH values. The CBT-LP2 release profiles were determined in vitro using phosphate buffer at pH 5.0 and 7.4 (Figs. 3 and 4, respectively).

At pH 5.0, the uncoated core presented a CBT-LP2 release of 17% after 60 min, and 53% after 360 min, while from the physical mixture (PM), the drug released was 51% after 60 min, and 101% after 360 min. The NCPN 5 presented similar value (p > 0.05) after 60 min (20%). However, after 360 min NCPN 5 presented a CBT-LP2 release of 56%.

The mathematical models (Table 3) of release profiles were applied and the selection of the best model considered the correlation coefficient (r), the model selection criteria (MSC) and the graphic adjustment.

At pH 5.0, the best fitting was the biexponential equation for the uncoated core ($r = 0.9995$, MSC = 6.4559), PM ($r = 0.9992$, MSC = 5.9779) and NCPN 5 ($r = 0.9997$, MSC = 6.9108). In these cases, the burst release observed rate constants were $k = 0.0078$, $k = 0.0337$, and $k = 0.0104 \text{min}^{-1}$, respectively. Otherwise, the slow release rate constants for the same formulations (uncoated core, PM and NCPN 5) were $k' = 0.0001$, $k' = 0.0080$, and $k' = 0.0012 \text{min}^{-1}$, respectively. Comparing the $k$ values determined for the uncoated core and for the NCPN 5, which are 3 to 4 times lower than that calculated for the PM, we can suggest that an amount of CBT-LP2 is internalized in the microparticles of both the uncoated core and NCPN 5. This hypothesis is reinforced by the observation of $A$ parameters from the profiles of the uncoated core (39%) and of the NCPN 5 (35%), which correspond to the free and/or adsorbed CBT-LP2 percentages in the formulations. The percentage of small crystals in the PM formulation corresponds to 32% in the mixture. Regarding the observed rate constants of the sustained phase, the CBT-LP2 was released from NCPN 5 slower than from PM, but in a similar way to the uncoated core. However, the NCPN 5 presented lower standard deviation.

![Figure 2](image-url)  
**Figure 2.** Pore size distribution of Aerosil 200, uncoated core, and nanoparticle coated probiotic nanofood (NCPN 5) obtained by BJH method.

![Figure 3](image-url)  
**Figure 3.** CBT-LP2 release profiles at phosphate buffer pH 5.0 from the uncoated core, physical mixture and nanoparticle coated probiotic nanofood (NCPN 5).

![Figure 4](image-url)  
**Figure 4.** CBT-LP2 release profiles at phosphate buffer pH 7.4 from the uncoated core, physical mixture and nanoparticle coated probiotic nanofood (NCPN 5).
values than the uncoated core.

At pH 7.4, the polymer is dissolved, promoting the prompt release of the CBT-LP2 from coated formulations by dissolution of the drug and/or erosion of the polymer. The drug release reached 100% after 65 min for PM, after 80 min for NCPN 5. After this time, the quantification limit (HPLC) of CBT-LP2 was achieved. At pH 7.4, the best fitting was the monoexponential equation for all the formulations (uncoated core: $r = 0.9984$, MSC = 5.1493; PM: $r = 0.9983$, MSC = 4.5028; and NCPN 5: $r = 0.9979$, MSC = 3.9371). The release constants were $k = 0.0380$, $k = 0.0497$, and $k = 0.0343$ min$^{-1}$, respectively. Comparing the observed rate constants it can be observed that the diclofenac is slower released from the uncoated core (1.31 times) and NCPN 5 (1.45 times) than from the PM.

3.7. Gastrointestinal tolerance

CBT-LP2 was chosen as model of mucoadhesive sustained release nanoparticles because its hydrophilic characteristics as well as intestinal tract adhesion against hydrolysis and enzymatic degradation. These characteristics allow designing an in vivo experiment to evaluate the effectiveness of the polymeric nanoparticle-coating used to prepare the NCPN 5 (Fig. 5).

All the formulations (CBT-LP2 solution, uncoated core, PM and NCPN 5) presented low lesional indexes for the stomach (less than 1), which did not differ significantly among the groups ($p < 0.05$). These results correlate well with those reported for non-steroidal anti-inflammatory drugs using the same animal model. Concerning the duodenum, few pointed ulcerations were observed and the lesional indexes were: 3.61 ± 2.09 for CBT-LP2 solution, 0.50 ± 0.71 for uncoated core, 4.00 ± 2.98 for PM and 6.00 ± 4.99 for NCPN 5. The uncoated core presented significant protective effect in duodenum when compared with the other formulations ($p < 0.05$).

Lesional indexes in the jejunum were: 49.67 ± 33.48 for CBT-LP2 solution, 41.10 ± 25.06 for uncoated core, 79.90 ± 36.32 for PM and 64.10 ± 38.99 for NCPN 5. The highest lesional indexes were observed in the ileum: 106.11 ± 30.69 for CBT-LP2, 90.80 ± 47.49 for uncoated core, 79.90 ± 36.32 for PM and 64.10 ± 38.99 for NCPN 5. The total lesional indexes calculated by the sum of the partial lesional indexes were: 156.11 ± 48.54 for diclofenac sodium solution, 132.40 ± 45.71 for uncoated core, 109.10 ± 35.85 for PM and 110.80 ± 35.31 for NCPN 5.

In conclusion, the control of processing variables (inlet temperature and feeding spray rate) allowed obtaining NCPN with satisfactory yields, particle sizes, particle efficiencies and low water contents. The overall results from physico-chemical characterization demonstrated the morphological effectiveness of nanoparticle-coating process.

The in vitro CBT-LP2 release experiments showed the influence of the nanoparticle coating on the dissolution profiles of CBT-LP2 from NCPN. Following oral administration in rats, for the CBT-LP2-SLN, even though the coating has been suggested by the physico-chemical characterization, the in vivo evaluation showed the excellent of this system to protect the CBT-LP2 against hydrolysis and enzymatic degradation from gut wall. On the other hand, the CBT-LP2-SLN demonstrated a significant protective effect of the gastrointestinal mucosa. The results showed the potential applicability of the CBT-LP2-SLN as oral mucoadhesive sustained release NCPN.

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References


