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Enhanced gastric tolerability and improved anti-obesity effect of capsaicinoids-loaded PCL microparticles

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ABSTRACT

Capsaicinoids show several pharmacological effects including weight loss. However, their pungency limits the long-term use through the gastrointestinal tract. In that sense, the goal of this study was to prepare capsaicinoids-loaded poly(ε-caprolactone) microparticles as an oral carrier in order to improve their gastric tolerability and to make feasible the long-term treatment of obesity. Formulations containing 3, 5 and 10% capsaicinoids were successfully obtained by simple emulsion/solvent evaporation method. Values of encapsulation efficiency above 90% were achieved. Microparticles showed spherical shape and smooth surface. The particle size was suitable for oral use in order to provide an extended release through the gastrointestinal tract. No chemical bond was observed between drug and polymer. Microencapsulation led to drug amorphization. Formulations prolonged the release of capsaicinoids without changing the release kinetic (biexponential model). Microencapsulation increased the gastric tolerability of capsaicinoids because it prevented inflammatory processes in the stomach of rats. Microparticles containing 5% capsaicinoids demonstrated a statistically significant reduction of Lee index, mesenteric and retroperitoneal fat pads of rats with obesity induced by hypothalamic lesion using monosodium L-glutamate. In summary, capsaicinoids-loaded poly(ε-caprolactone) microparticles are low-irritative oral controlled-release carriers for a long-term use in obesity.

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1. Introduction

Capsaicin (8-methyl-*N*-vanillyl-*trans*-6-nonenamide) and dihydrocapsaicin (8-methyl-*N*-vanillylnonanamide) (Fig. 1) are the two most pungent capsaicinoids from chili peppers (*Capsicum* spp.). These capsaicinoids exert multiple pharmacological and physiological effects including analgesic, anticancer, anti-inflammatory, antioxidant and anti-obesity activities. Therefore capsaicinoids have a potential value in clinic for pain relief, cancer prevention and weight loss [1].

A current systematic review reported that consumption of capsaicinoids increases energy expenditure by about 50 kcal/day which produces a clinically significant weight loss in 1–2 years [2]. It was also observed that a regular use of capsaicinoids significantly reduced abdominal adipose tissue levels and decreased appetite and energy intake [2]. In addition, the mechanism of action of capsaicinoids on obesity has been extensively studied over the past decade. Capsaicin suppresses obesity-induced inflammatory responses by reducing the levels of TNFα, IL-6, and MCP-1, and enhances adiponectin levels in adipose tissue and liver which are important for insulin response [3,4]. These beneficial effects are associated with the dual action of capsaicin on PPARγ/PPARα and TRPV-1 expression/activation related to NF-κB inactivation and PPARγ activation [4]. Moreover, capsaicin suppresses macrophage migration induced by the adipose tissue and macrophage activation. Capsaicin significantly reduced palmitate-induced MIP-1 and IL-8 gene expressions as well as palmitate-stimulated activation of JNK, c-Jun, and p38 which improved β-oxidation [5]. Furthermore the induction of apoptosis in 3T3-L1 preadipocytes by capsaicin is mediated through the activation of caspase-3, Bax, and Bak and through the cleavage of PARP and the down-regulation of Bcl-2 [6].

In spite of these pharmacological properties and their biomolecular effects, capsaicinoids are very irritating substances causing pain and burning in low concentrations on skin and mucosae. Moreover, a long-term use on skin or through the gastrointestinal tract is limited due to pungency of capsaicinoids [7]. In particular, the pungency of capsaicinoids may induce increased salivation, gastric secretion, and gastrointestinal disorders when administered orally [8].

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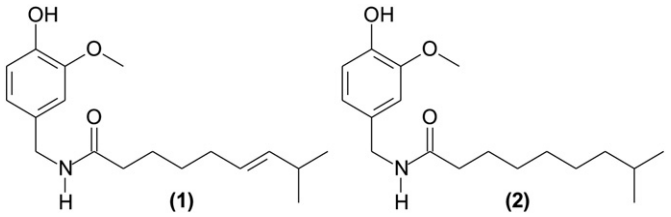

Fig. 1. Chemical structures of capsaicin (1) and dihydrocapsaicin (2).

In order to minimize these side effects and/or to control the drug release, some recent papers have been demonstrated the feasibility of incorporating capsaicinoids, mainly capsaicin, in different release carriers. Hydrogels of PEG-PLGA-PEG and multilamellar liposomes for treating cystitis [9], inclusion complexes using hydroxypropyl-β-cyclodextrin for improving percutaneous absorption of capsaicin [10], capsaicin-loaded adhesive for reducing pain of herpetic neuralgia [11], capsaicin immobilized onto hyperbranched poly(amidoamine)-grafted silica for having biorepellent activity [12], capsaicin-loaded nanoemulsions stabilized by layer-by-layer self-assembly [13], nanocapsules containing capsaicin and dihydrocapsaicin for prolonging the topical analgesia and reducing the burning effect [14], capsaicin-loaded poly(lactic-*co*-glycolic acid) nanoparticles for extending the drug release [15], niosomes and microemulsions containing capsaicin for topical administration [16], and lipid-polymer hybrid nanoparticles loading both capsaicin and anti-TNFα siRNA for inhibiting skin inflammation [17] were previously reported. However, no previous paper was devoted to investigate polymeric microparticles containing capsaicinoids in order to achieve a low irritative effect for a long-term use through the gastrointestinal tract.

PCL has drawn extensive attention with regard to tissue regeneration and controlled-release drug applications. Its good solubility, low melting point (59–64 °C) and exceptional drug-compatibility have made PCL a continuous research focus in drug delivery area [18]. In addition, this synthetic polyester shows suitable biocompatibility and biodegradability [19] for obtaining low-irritative oral preparation. In that sense, PCL was chosen as a promising controlled-release carrier for capsaicinoids. Moreover, there is a lack of data concerning the effect of these capsaicinoids-loaded PCL microparticles on obesity which may open up new opportunities for its suitable treatment.

Thus, the aim of this paper was to obtain capsaicinoids-loaded PCL microparticles by simple emulsion/solvent evaporation method and to evaluate both their gastric tolerability and anti-obesity potential in order to explore the feasibility of applying these polyester microparticles as an oral drug delivery carrier for controlled release.

2. Materials and methods

2.1. Materials

Capsaicinoids were purchased from Valdequímica Produtos Químicos (São Paulo, Brazil) as a mixture of 73.1% capsaicin and 26.9% dihydrocapsaicin. Poly-ε-caprolactone (PCL) (Mw 70 000–90 000 g/mol, Sigma-Aldrich, St. Louis, MO, USA) and poly(vinyl alcohol) (PVA) (Mw 72 000 g/mol, 88.5 mol% of hydrolysis, Vetec, Rio de Janeiro, Brazil) were used as received. The other reagents and solvents were of analytical/HPLC grade.

2.2. Methods

2.2.1. Preparation of capsaicinoids-loaded PCL microparticles

The microparticles containing capsaicinoids were obtained by the simple emulsion/solvent evaporation method [19]. Three formulations (Table 1) were prepared depending on the amount of capsaicinoids into their compositions (3, 5 and 10%). Briefly, the organic phase was added into the aqueous phase under mechanical stirring (3500 rev/min, RW 20 digital overhead paddle stirrer, IKA Works, Wilmington, NC, USA) for 5 min. The emulsion was kept under mechanical stirring (1000 rev/min, RW 20 digital overhead paddle stirrer, IKA Works, Wilmington, NC, USA) at room temperature for 6 h. After evaporating organic solvent, microparticles were separated by centrifugation (2500 rev/min, 10 min, BE-4004 centrifuge, Bio Eng, São Paulo, Brazil), washed twice with purified water and dried under vacuum at 40 ± 1 °C (TE-395 vacuum oven, Tecnal, Piracicaba, Brazil) for 6 h. The samples were stored into a desiccator under vacuum at room temperature. All formulations were obtained in triplicate. Unloaded-microparticles were also prepared as negative control.

Table 1
Composition of PCL microparticles.

Composition	Formulation			
	MC0	MC3	MC5	MC10
Aqueous phase				
Polysorbate 80 (g)	0.25	0.25	0.25	0.25
PVA (g)	4.00	4.00	4.00	4.00
Purified water (mL)	200.0	200.0	200.0	200.0
Organic phase				
Capsaicinoids (g)	–	0.06	0.10	0.20
PCL (g)	2.00	1.94	1.90	1.80
Methylene chloride (mL)	40.0	40.0	40.0	40.0

2.2.2. Moisture content

The water content of capsaicinoids, PCL and microparticles was performed using an infrared moisture analyzer (Top Ray 160, Bel Engineering, Monza, Italy). For each sample, an amount of 1.000 g was placed on an aluminum plate and dried at 105 °C until constant weight. The percentage corresponding to the mass loss was determined as moisture content. The tests were carried out in triplicate.

2.2.3. Drug loading and encapsulation efficiency

An amount of microparticles, equivalent to 3 mg of capsaicinoids, was weighted and magnetic stirred (1000 rev/min, 752A magnetic stirrer, Fisatom, São Paulo, Brazil) with 7 mL ethanol for 24 h in order to completely extract the drug. The volume was made up to 10 mL and filtered through a poly(vinylidene fluoride) membrane filter (Durapore membrane, 0.45 μm pore size, Millipore, Bedford, MA, USA) for quantification. After suitable dilution in ethanol, the concentration of capsaicinoids was determined by HPLC system (Merck-Hitachi LaChrom D-7000 HPLC system, Darmstadt, Germany) using a LiChrospher RP-18 analytical column (250 × 4.6 mm, 5 μm) at 30 °C with UV detection at 280 nm in triplicate. The mobile phase used was acetonitrile:water (70:30), pH 4.5, adjusted with acetic acid at a flow rate of 0.75 mL/min. The validation of this HPLC method was previously performed through the following parameters: linearity, specificity, limit of detection, limit of quantification, accuracy, precision, and robustness [20]. The concentration range varied from 10 to 50 μg/mL. Linearity was 0.9996 and the detection limit was 56.04 ng/mL. The encapsulation efficiency (EE) was obtained using Eq. (1).

$$EE = \left(\frac{\text{mass of capsaicinoids in microparticles}}{\text{theoretical mass of capsaicinoids}}\right) \times 100. \quad (1)$$

2.2.4. Scanning electron microscopy

The samples were mounted on aluminum stubs, sputtered with gold and analyzed using a scanning electron microscope (JSM-6360LV, Jeol, Kyoto, Japan) at an accelerating voltage of 10 or 15 kV with different magnifications.

2.2.5. Particle size and size distribution

The particle size and size dispersion of PCL microparticles were measured by laser diffraction spectrometry in a Cilas 920 L apparatus (Marseille, France). The dried powder samples were suspended in filtered water and sonicated into the ultrasonic bath coupled to the equipment for 1 min before measurements. Then, the mean diameters ± standard deviations and the size distributions were determined. The span was calculated using Eq. (2).

$$\text{span} = \frac{d_{(v,90)} - d_{(v,10)}}{d_{(v,50)}} \quad (2)$$

where $d_{(v,10)}$, $d_{(v,50)}$, and $d_{(v,90)}$ are the particle diameters determined at the 10th, 50th, and 90th percentile of the undersized particle distribution curve, respectively.

2.2.6. Fourier-transformed infrared spectroscopy

The Fourier-transformed infrared spectra of capsaicinoids, PCL, microparticles and physical mixture (1:1 capsaicinoids:PCL) were recorded from 4000 to 400 cm^{-1} on a Shimadzu IR Prestige-21 spectrophotometer (Kyoto, Japan) using KBr pellets with 32 scans and resolution of 4 cm^{-1}.

2.2.7. X-ray powder diffraction

Wide-angle X-ray powder diffraction was performed with a Rigaku X-ray diffractometer (Rigaku Ultima IV, Shibuya-Ku, Japan). The 2θ was increased from 5° to 50° at a scan rate of 2°/min using a Cu-Kα source (λ = 1.5418 Å) at 40 kV and 30 mA.

2.2.8. Differential scanning calorimetry (DSC)

DSC curves of capsaicinoids, PCL, physical mixture and microparticles were obtained in a DSC-200 F3 calorimeter (Netzsch, Burlington, VE, USA) using aluminum crucibles with 2.5 ± 0.1 mg of sample under dynamic N_2 atmosphere (flow rate: 50 mL/min). The temperature range was from −100 °C to 120 °C with a heating rate of 10 °C/min. An empty aluminum pan was used as reference. The DSC cell was calibrated with indium (m.p. = 156.6 °C; ΔH_{fusion} = 28.54 J/g) and zinc (m.p. = 419.6 °C).

2.2.9. In vitro drug release

In vitro release was carried out for pure drug and capsaicinoids-loaded PCL microparticles. Dissolution assays were performed in a Nova Ética dissolution tester (299/3, Vargem Grande Paulista, Brazil) equipped with paddles (apparatus II) in 900 mL of degassed phosphate buffer solution (pH 6.8, 50 mmol/L KH_2PO_4 and 22.4 mol/L NaOH) for 36 h. System was kept at a thermostatically controlled temperature of 37 ± 0.5 °C and stirred at 75 rev/min. At predetermined time intervals, samples were collected (10 mL), filtered (0.45 μm pore size) and spectrophotometrically evaluated (Genesys 10S spectrophotometer, Thermo Scientific, Madison, WI, USA) at 210 nm. The dissolution value was obtained from the amount of drug released. A correction factor was applied to the cumulative dilution caused by replacement of the sample with an equal volume of fresh medium. Three dissolution tests were carried out for each batch of microparticles. Considering that all formulations were obtained in triplicate, a total of nine dissolution experiments were performed for each capsaicinoids-loaded PCL microparticle.

2.2.10. Analysis of release behavior

Dissolution profiles of capsaicinoids and capsaicinoids-loaded PCL microparticles were compared by independent and dependent methods. As model-independent analysis, dissolution efficiency, the area under a dissolution curve between defined time points, was used. Profiles were also investigated by model-dependent methods using the MicroMath Scientist 2.01 software (Salt Lake City, UT, USA). Data were tested to fit first-order, biexponential, zero-order, and Weibull equations [19]. The selection of model-dependent method was based on the best correlation coefficient (r), the best model selection criteria (MSC), and the best graphic adjustment.

In order to have some insight into the drug release mechanism, a very simple and semi-empirical equation to describe drug release from polymeric systems, the power law (Korsmeyer–Peppas model) [21], was also applied (Eq. (3)).

$$ft = at^n \quad (3)$$

where ft is the drug dissolved fraction at time t, n is the release exponent, indicative of the mechanism of the drug release and a is the constant incorporating structural and geometric characteristics of the drug dosage form.

2.2.11. In vivo biological assays

All experimental protocols were performed in accordance to the Guide for the Care and Use of Laboratory Animals [22] published by the U.S. National Institutes of Health (NIH) and were approved by the Ethics Committee on Animal Use of the State University of Ponta Grossa (CEUA/UEPG) under protocol number 14/2012.

2.2.11.1. Gastric tolerability

2.2.11.1.1. Animals. Male Wistar rats, 60 days old, weighing between 180 and 230 g, were housed at room temperature (22 ± 2 °C) with controlled 12:12 h light/dark cycle. Standard rodent chow and water were always available except during the experiments.

2.2.11.1.2. Experimental protocol. Thirty rats were randomly divided into five groups of six animals each one. Group 1 received pure capsaicinoids (30 mg/kg, orally), group 2 received capsaicinoids-loaded PCL microparticles MC5 (amount equivalent to 30 mg of capsaicinoids/kg, orally), group 3 received unloaded-microparticles MC0 (equivalent mass amount to group 2, orally), group 4 received vehicle (medium chain triglycerides, 1 mL) and group 5 received control (sterile saline, 1 mL). The treatment was carried out during 15 consecutive days by a gastric gavage once-a-day. Animals were then sacrificed using an anesthetic overdose for further analyses.

2.2.11.1.3. Histological analysis. Each stomach was removed through an abdominal incision, immediately washed with distilled water twice, and transferred to a vessel containing Bouin's fixative. For histological analysis, each stomach was processed, embedded in paraffin, sectioned to a thickness of 5 μm and stained with hematoxylin and eosin for microscopic examination [23].

2.2.11.2. Obesity induced by hypothalamic lesion using monosodium L-glutamate (MSG)

2.2.11.2.1. Animals and induction of MSG obesity. Neonate male Wistar rats were subcutaneously injected, during the first 5 days of life, with MSG at a dose of 4 g/kg. Control animals received equimolar saline solution. Both animal groups were weaned when 21 days old. All animals were housed, under control conditions, in a 12:12 h light/dark cycle and temperature (22 ± 2 °C). Water and standard rodent chow were supplied ad libitum.

2.2.11.2.2. Experimental protocol. Thirty MSG-treated rats, 60 days old, each one was randomly divided into five groups of six animals. Group 1 received pure capsaicinoids (15 mg/kg, orally), group 2 received capsaicinoids-loaded PCL microparticles MC5 (amount equivalent to 15 mg of capsaicinoids/kg, orally), group 3 received unloaded-microparticles MC0 (equivalent mass amount to group 2, orally), group 4 received vehicle (medium chain triglycerides, 1 mL) and group 5 received control (sterile saline, 1 mL). The same procedure was performed to thirty untreated rats (control animals), each one was randomly divided into five groups of six animals. These treatments were carried out during 30 consecutive days by a gastric gavage once-a-day. When 90 days old, MSG-treated and untreated rats were

Table 2
Water content[a], capsaicinoids entrapped into microparticles[a], encapsulation efficiency (EE)[a], particle size[a], and *span* for PCL microparticles.

Formulation	Water content (%)	Capsaicinoids-loaded ($mg \cdot g^{-1}$)	EE (%)	Mean diameter (μm)	*Span*
MC0	0.86 ± 0.05	–	–	8.99 ± 7.33	1.93
MC3	1.15 ± 0.09	27.77 ± 1.08	92.57 ± 3.60	9.29 ± 6.21	1.98
MC5	0.78 ± 0.08	47.65 ± 2.11	95.30 ± 4.22	9.07 ± 4.72	1.79
MC10	1.00 ± 0.09	95.23 ± 1.52	95.23 ± 1.52	11.97 ± 7.40	1.85

[a] Mean (n = 3) ± standard deviation.

weighed, anesthetized with ketamine and xylazine (55 and 8 mg/kg, respectively) and slaughtered by cervical dislocation [24].

2.2.11.2.3. Evaluation of obesity. The naso-anal length (cm) was then measured in order to calculate Lee index (Eq. (4)), a predictor of obesity in rodents [24].

$$\text{Lee index} = \left(\frac{\sqrt[3]{\text{weight}(g)}}{\text{naso-anal length}(\text{cm})}\right) \times 1000. \quad (4)$$

Mesenteric, periepididymal, and retroperitoneal fat pads were removed, washed and weighed to estimate the effect of treatments in obese and control rats.

2.2.12. Statistical analysis

Results were expressed as mean ± standard error of mean. Comparisons among groups were tested by one-way ANOVA with Bonferroni's *post-hoc* test. The significance level was set at $\alpha = 5\%$ ($p < 0.05$). The analyses were performed using the GraphPad Prism software (version 5.00, San Diego, CA, USA).

3. Results and discussion

The capsaicinoids-loaded PCL microparticles were successfully prepared by the simple emulsion/solvent evaporation method. After drying, all the obtained formulations showed powder aspect and white color similar to PCL. Capsaicinoids and PCL presented water content of 0.19 ± 0.03% and 1.07 ± 0.06%, respectively. Unloaded and capsaicinoid-loaded microparticles demonstrated only residual moisture values as summarized in Table 2. It was observed that formulations had water content similar or lower than pure polymer which demonstrated that vacuum drying was able to remove the water used during microencapsulation. Furthermore, these values are consistent with earlier reports that obtained microparticles by emulsion/solvent evaporation method. Puthli and Vavia [25] prepared polymeric microparticles containing levonorgestrel and obtained low residual water contents. Mendes and colleagues [19] developed resveratrol-loaded PCL microparticles and achieved moisture values lower than 1.57%.

3.1. Drug loading and encapsulation efficiency

Table 2 shows the results for drug content and encapsulation efficiency (EE). All formulations presented suitable EE values higher than 90% corresponding to drug loadings close to the theoretical concentrations. These data can be strongly related to the low aqueous solubility of capsaicinoids (capsaicin has solubility of 10.3 mg/L in water at 25 °C [26]) which provides high drug entrapment into PCL microparticles. In addition, these results are similar to those previously reported in which Eudragit RS100 nanocapsules containing capsaicinoids were prepared by the interfacial deposition procedure and presented EE close to 100% [14].

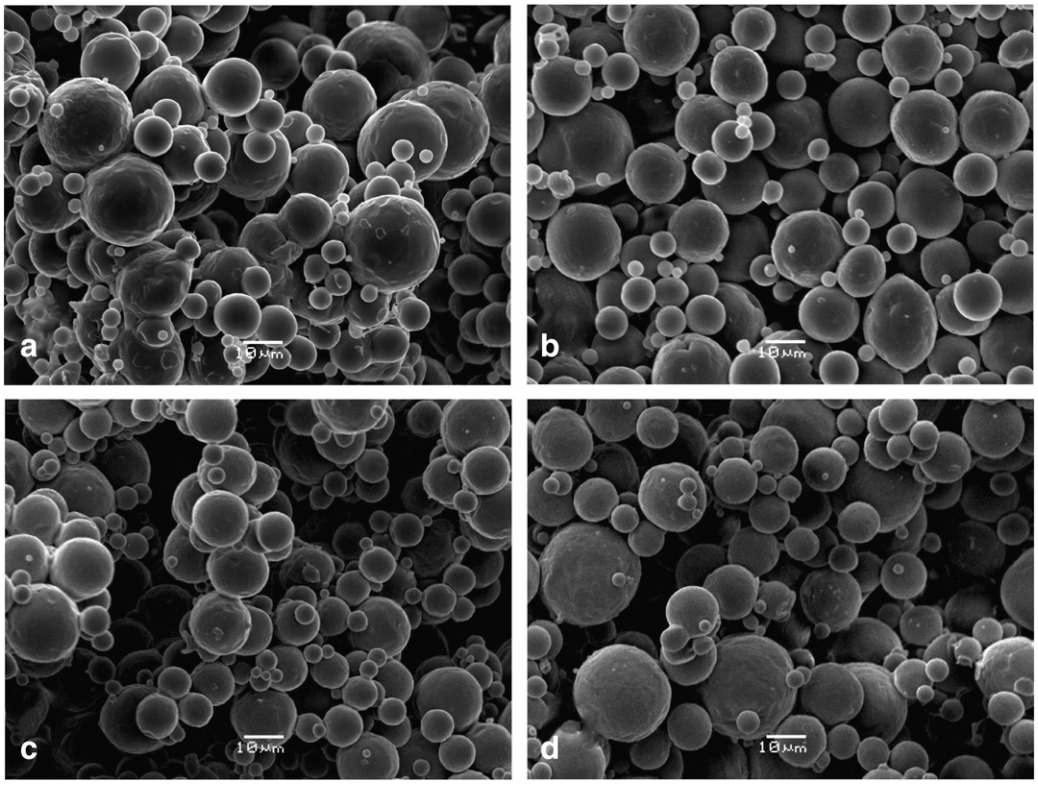

Fig. 2. Scanning electron micrographs of PCL microparticles: MC0 (a), MC3 (b), MC5 (c), and MC10 (d) (magnification: 1000×).

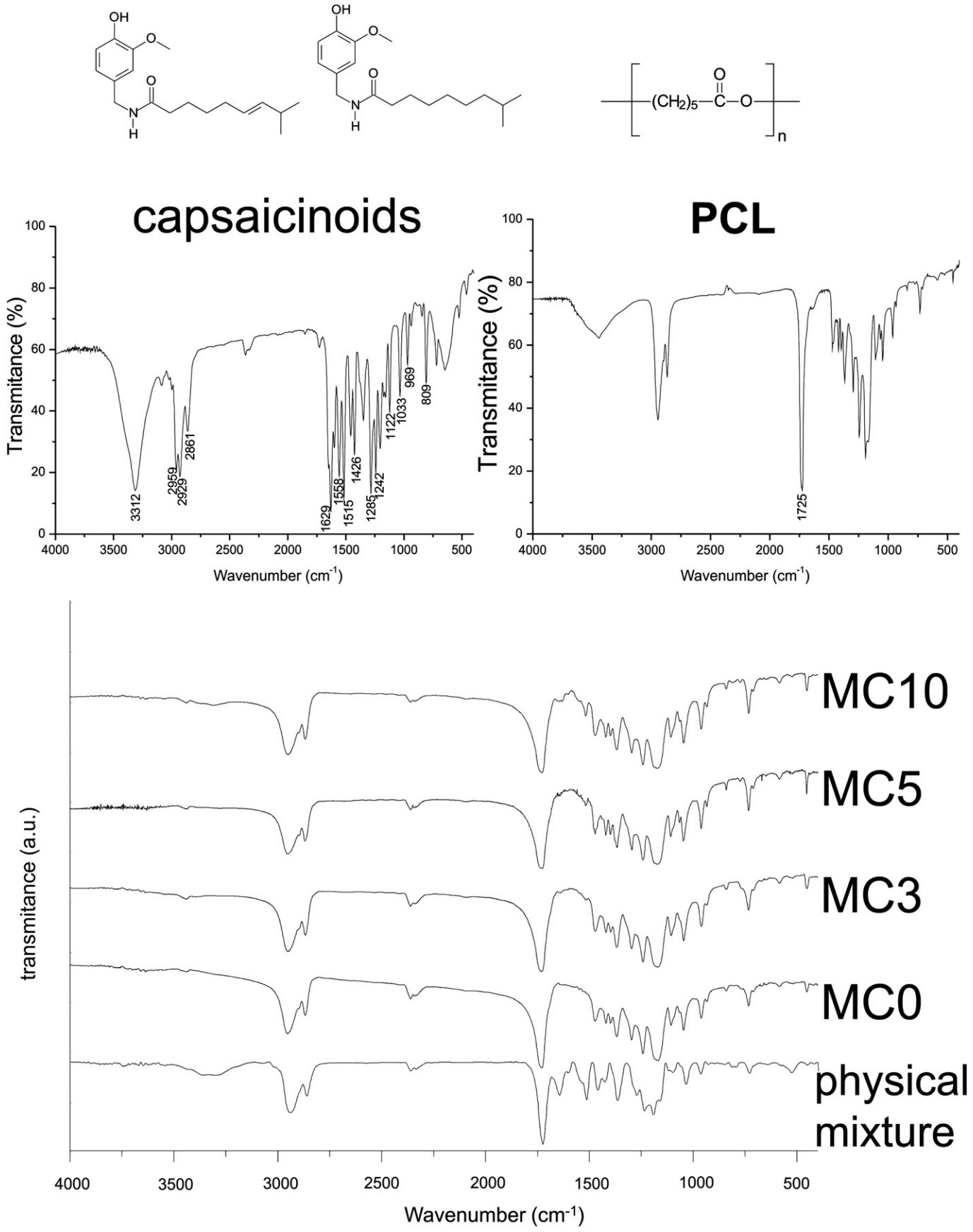

Fig. 3. FTIR spectra of capsaicinoids, PCL, physical mixture, and PCL microparticles.

3.2. Scanning electron microscopy

Scanning electron microscopy analysis was performed in order to investigate the shape and surface of microparticles. PCL microparticles were spherical in shape and had a smooth surface (Fig. 2). No pore was observed on the surface of all formulations. The same morphological data were verified in the other previous studies that obtained PCL microparticles by the simple emulsion/solvent evaporation technique

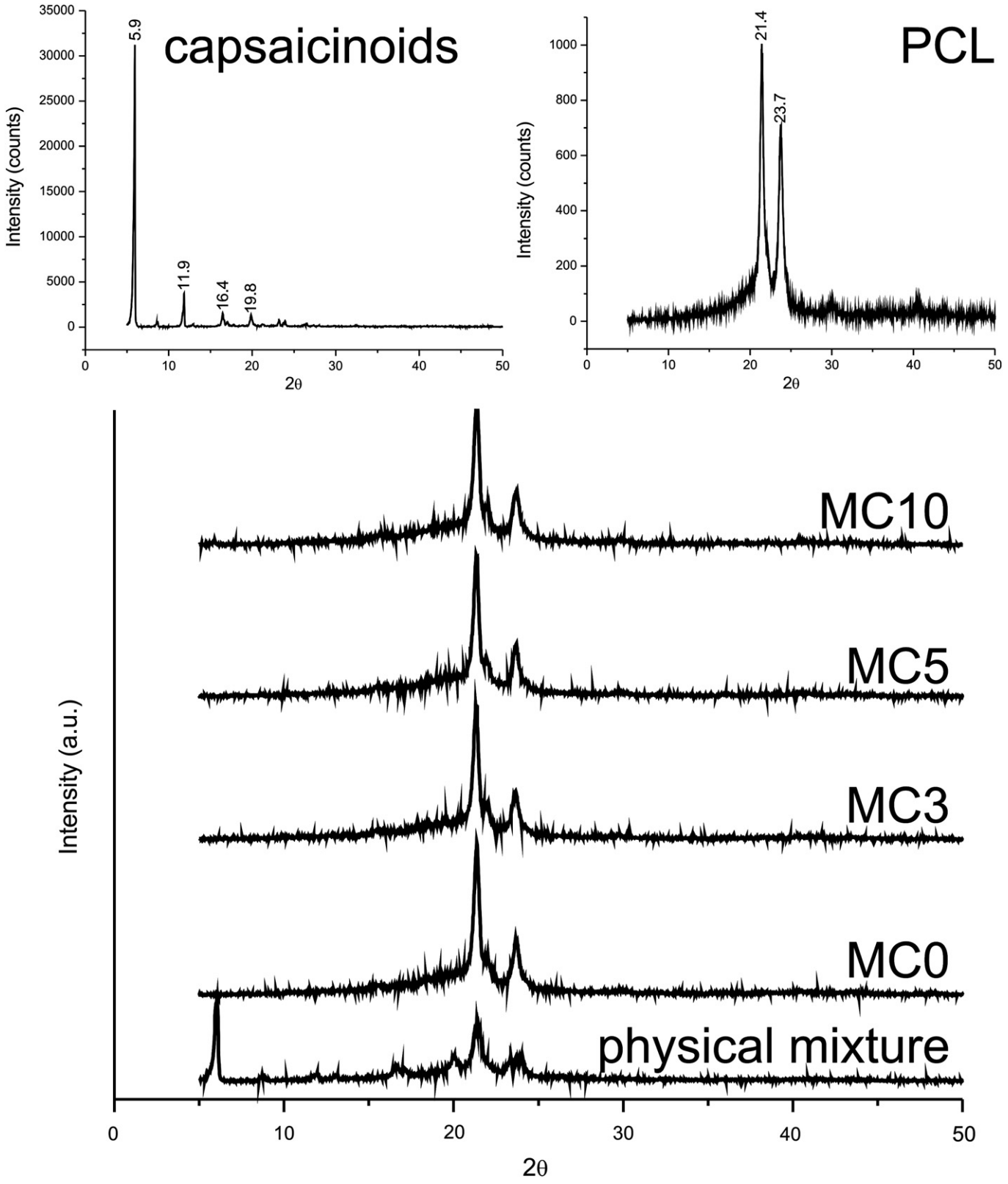

Fig. 4. XRPD patterns of capsaicinoids, PCL, physical mixture, and PCL microparticles.

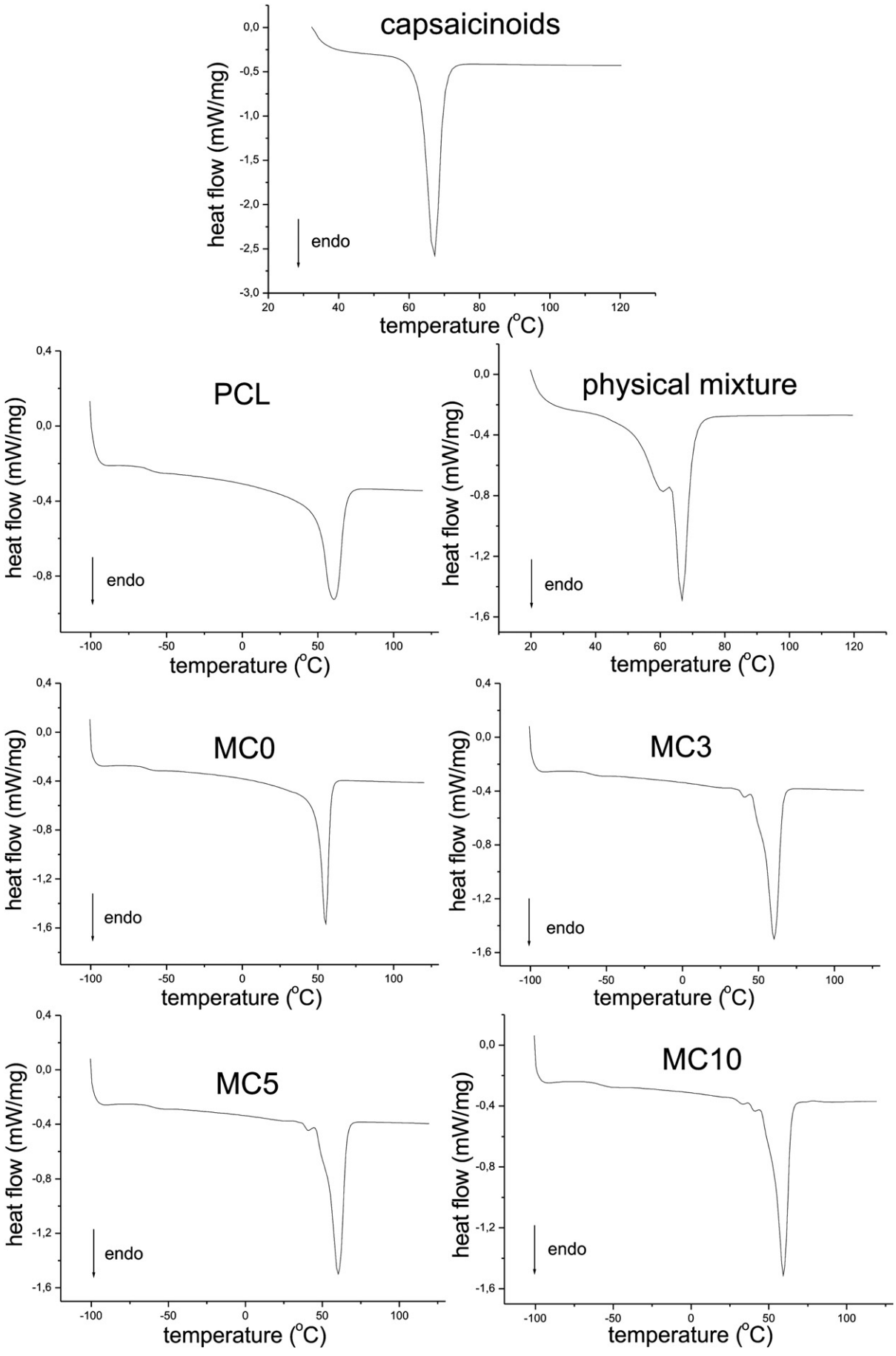

Fig. 5. DSC curves of capsaicinoids, PCL, physical mixture, and PCL microparticles.

[19,27]. Unloaded-microparticles had similar morphology and size which indicate an absence of detrimental effects due to the drug loading.

3.3. Particle size and size distribution

For determining the particle size and size distribution, granulometric analyses of PCL microparticles were carried out and are indicated in Table 2. All formulations presented mean diameter values under 12 μm. These micrometer-sized particles are suitable for oral administration since they do not allow an uptake by enterocytes and lead to an extended bowel transit [28]. All PCL microparticles revealed *span* value below 2 which represents a narrow dispersion around mean size and suggests an adequate unimodal behavior.

3.4. Fourier-transformed infrared (FTIR) spectroscopy

Chemical interactions between drug and polymers commonly lead to identifiable changes in FTIR patterns. In that sense, FTIR spectra were achieved in order to explore whether the microencapsulation resulted in any chemical change by comparing differences in band assignments among raw materials and formulations. FTIR spectra recorded for capsaicinoids, PCL, physical mixture and PCL microparticles are represented in Fig. 3. The FTIR spectrum of pure capsaicinoids showed assignments for both capsaicin and dihydrocapsaicin at 3312 cm^{-1} (broadened O–H and N–H stretch), 2959 cm^{-1}, 2929 cm^{-1}, and 2861 cm^{-1} (aliphatic C–H stretch), 1629 cm^{-1} (olefinic C═C stretch, C═O stretch, amide II), 1600 cm^{-1} and 1558 cm^{-1} (aromatic C═C stretch), 1515 cm^{-1} (N–H bend and C–N stretch, amide II), 1426 cm^{-1} (C–H bend), 1285 cm^{-1} (asym C–O–C stretch), 1242 cm^{-1} (C–N stretch), 1204 cm^{-1} (C–O stretch), 1122 cm^{-1} (C–N stretch), 1033 cm^{-1} (C–O stretch), 969 cm^{-1} (*trans*, H–C═C bend, capsaicin only), and 809 cm^{-1} (out-of-plane C–H bend). PCL infrared spectrum exhibited a strong band at 1725 cm^{-1} (ester C═O stretch). Other typical bands at 2943 cm^{-1} (asym C–H_2 stretch), 2864 cm^{-1} (sym C–H_2 stretch), 1060–1150 cm^{-1} (asym C–O–C stretch), and 800–975 cm^{-1} (sym C–O–C stretch) were observed.

Considering their FTIR spectra, capsaicinoids-loaded PCL microparticles and physical mixture presented band assignments at the same wavenumber range. Therefore it is possible to suggest that no chemical bond between drug and polymer was formed during microencapsulation keeping the expected therapeutic effect of capsaicinoids.

3.5. X-ray powder diffraction (XRPD)

X-ray powder diffraction analysis was performed for investigating the crystallinity of the obtained formulations in comparing raw materials. Fig. 4 summarizes the XRPD patterns of capsaicinoids, PCL, physical mixture and PCL microparticles. Pure drug showed intense diffraction peaks at Bragg angles $2\theta = 5.9°$ (24 000), 11.9° (4500), 16.4° (1240), and 19.8° (1870) which are indicative of the crystalline structure of capsaicinoids. For PCL, two diffraction peaks at Bragg angles $2\theta =$ 21.4° (1005) and 23.8° (715) were observed which were attributed to the diffraction of the (110) lattice plane and the (200) lattice plane of the semi-crystalline PCL, respectively [18]. All formulations demonstrated crystalline diffraction patterns similar to pure PCL. These results indicate that the microencapsulation procedure provided a remarkable decrease of the crystalline diffraction peaks of capsaicinoids leading to drug amorphization. The preparation of PCL microparticles containing capsaicinoids as amorphous solids, when compared to the original crystalline structure of the drug, may facilitate the dissolution process which is desirable mainly due capsaicinoids have low solubility in aqueous media [26].

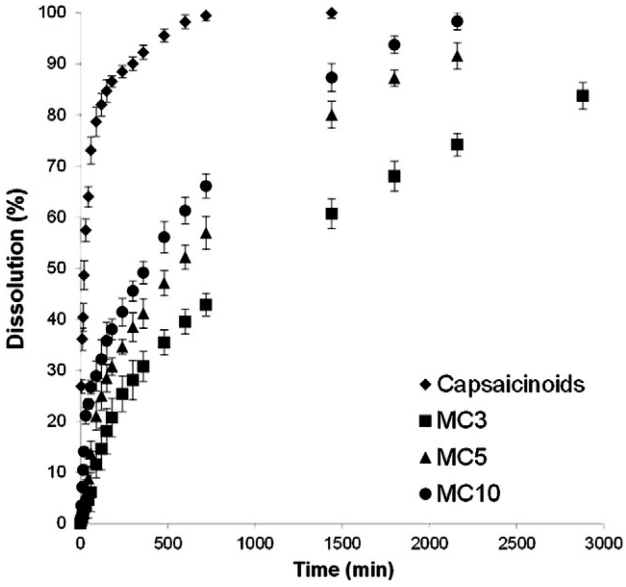

Fig. 6. In vitro dissolution profiles of pure capsaicinoid and drug-loaded PCL microparticles. All formulations were obtained in triplicate and a total of nine dissolution experiments were performed for each capsaicinoids-loaded PCL microparticles.

3.6. Differential scanning calorimetry (DSC)

The thermal behavior of formulations was investigated by analyzing the thermograms of capsaicinoids, PCL, physical mixture and PCL microparticles that are displayed in Fig. 5. Capsaicinoids showed a melting event at 67.2 °C in accordance to the literature [29]. PCL presented glass transition temperature and melting temperature at −60.9 and 61.0 °C, respectively, confirming previously reported data [19]. As expected, the thermogram of physical mixture demonstrated endothermic events attributed to both polymer and drug. However, PCL microparticles showed only one melting event at 61.9, 61.4, 60.4, and 59.2 °C for MC0, MC3, MC5, and MC10, respectively. Therefore the typical melting event of capsaicinoids was not observed in DSC curves of drug-loaded PCL microparticles. This thermal behavior indicates that a drug amorphization occurred and reinforces the results verified by XRPD.

3.7. In vitro drug release

In order to verify whether PCL microparticles were able to control capsaicinoid release, the dissolution profiles of the formulations were compared to the pure drug. Fig. 6 depicts the results of the in vitro drug release experiments. The mean time for 80% release of pure capsaicinoids was 105 min. However, formulations demonstrated the mean dissolution times of 2550 min (MC3), 1440 min (MC5), and 1125 min (MC10) for 80% drug release. Therefore capsaicinoids from PCL microparticles showed a slower dissolution rate than pure drug. These results demonstrate that PCL played an important role on the delay of drug dissolution. In addition, formulation MC3 (3% capsaicinoids) showed slower release

Table 3
Release data obtained by fitting the dissolution profiles of pure capsaicinoid and capsaicin-loaded PCL microparticles to the biexponential equation.

Material	Biexponential model			
	MSC	r	α (min^{-1})	β (min^{-1})
Capsaicinoids	2.06	0.9961	0.06026	0.004914
MC3	6.43	0.9994	0.00708	0.000533
MC5	5.67	0.9988	0.01446	0.000961
MC10	5.70	0.9990	0.04662	0.001235

of capsaicinoids. Probably, the higher amount of polyester in this formulation had a remarkable effect in controlling the drug release rate.

3.8. Analysis of release behavior

Microencapsulation led to a remarkable decrease of dissolution efficiency (DE) values. Whereas pure capsaicinoids presented DE of 97.0% along 2880 min, PCL microparticles provided DE of 56.6% (MC3), 72.0% (MC5), and 79.0% (MC10) at the same time interval which is an indicative of a controlled release behavior. Moreover, the dissolution profiles were fitted to mathematical models, and the selection of the best model considered the correlation coefficient (r), the model selection criteria (MSC), and the graphic adjustment. Capsaicinoids and loaded-PCL microparticles were better fitted to the biexponential equation than other models. The burst-release apparent rate constant (α) and the slow-release apparent rate constant (β) for pure capsaicinoids and formulations are summarized in Table 3.

These results demonstrated that PCL microparticles were able to reduce the drug dissolution rate, nevertheless without changing its release model. The first stage of release was initially rapid (burst release) whereas the second stage of release was slow (controlled release). This behavior is very interesting for the desired therapeutic purpose since the burst release may help to reach the effective concentration of capsaicinoids rapidly in plasma, whereas the controlled release might maintain the effective concentration of drug in plasma for a long time [30].

Regarding the mathematical modeling fitting the Korsmeyer–Peppas model, PCL microparticles showed n values of 0.58 (MC3), 0.56 (MC5), and 0.42 (MC10). Formulations MC3 and MC5 demonstrated diffusion exponent (n) ranged between 0.43 and 0.85 indicating an anomalous transport [21]. Therefore both diffusion and erosion

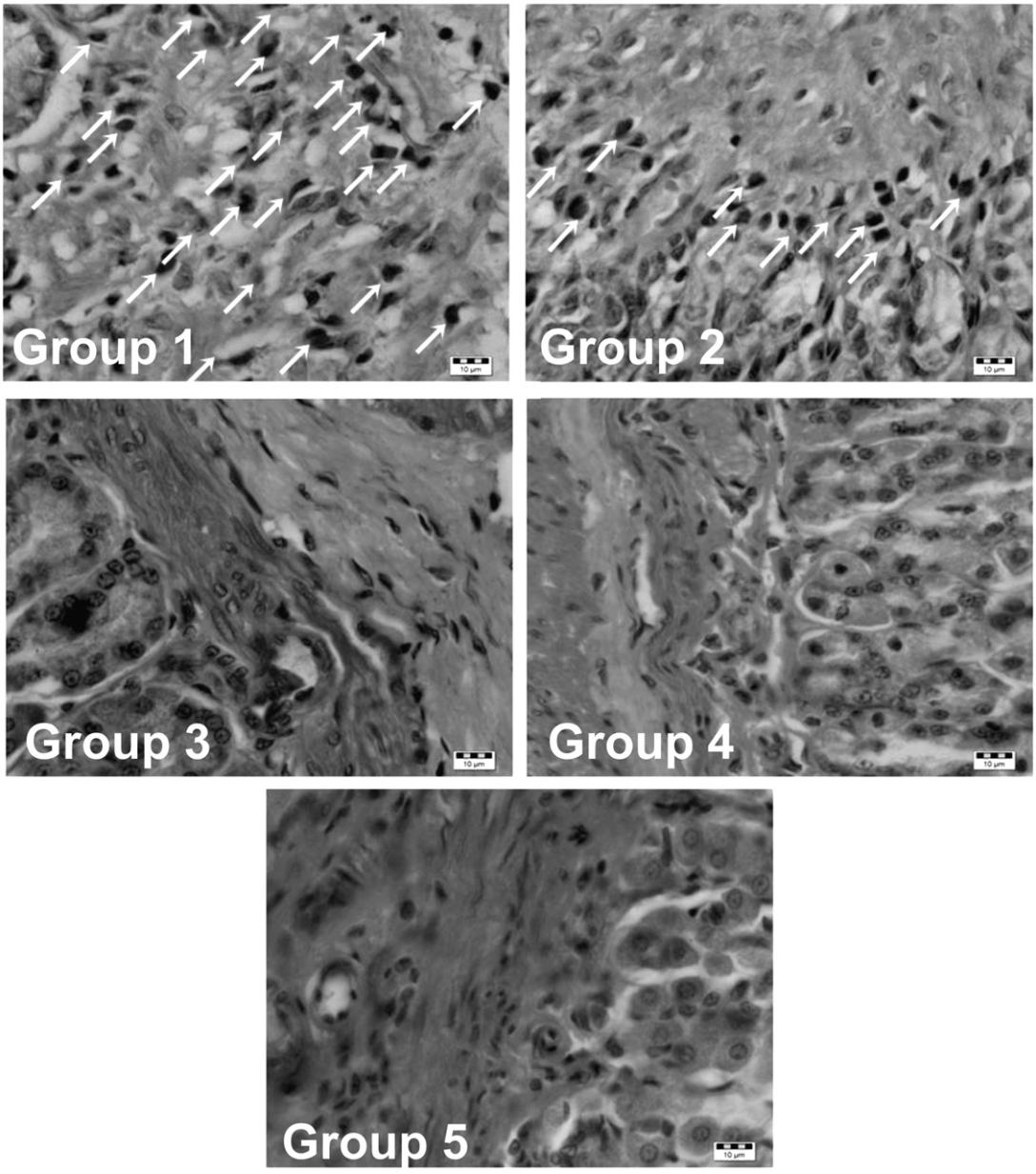

Fig. 7. Histological analysis of rat stomachs demonstrating cellular infiltration in the submucosal layer using capsaicinoids (Group 1) and capsaicinoids-loaded PCL microparticles MC5 (Group 2) after the 15-day treatment. No histological change was observed for unloaded-microparticles MC0 (Group 3), vehicle (Group 4), and saline (Group 5). Images are representative of transverse sections of rat stomachs stained with hematoxylin-eosin (magnification: 1000×, scale bar: 10 μm).

mechanisms play role in capsaicinoids release from PCL matrix. However formulation MC10 presented *n* value lower than 0.43 which denotes that the release of the drug followed Fickian diffusion kinetics [21].

Taking all these results in account, it is possible to suggest that the capsaicinoids-loaded PCL microparticles offer a feasible system to control capsaicinoid release along the gastrointestinal tract.

3.9. In vivo biological assays

In order to investigate the gastric tolerability and the effect of capsaicinoids-loaded PCL microparticles in obese rats induced by neonatal administration of monosodium L-glutamate (MSG), formulation MC5 was chosen for further in vivo pharmacological evaluations due to the lowest water content (0.78%), the higher encapsulation efficiency (95.3%), a narrow size dispersion (*span* = 1.79), and a release of 80% of capsaicinoids during 24 h.

3.9.1. Gastric tolerability

The gastric tolerance test was performed in order to explore whether the microencapsulation may avoid histological damages in the stomachs of rats when a high amount of capsaicinoids (30 mg/kg) was used. This dose was chosen based on a previous study that investigated the tissue distribution and elimination of capsaicin in rats after oral administration [31]. The authors reported that 30 mg/kg is an amount five times greater than the average daily intake among Asian people that usually have a high consumption of peppers.

After the 15-day treatment, group 1 that received pure capsaicinoids showed infiltration of polymorphonuclear leukocytes in the submucosal layer of stomach (Fig. 7) which is indicative of inflammatory lesion. However, only a mild leukocyte infiltration was observed in group 2 that received formulation MC5 containing the same amount of capsaicinoids. These results demonstrate that microencapsulation was able to increase the gastric tolerability of capsaicin and dihydrocapsaicin for preventing the inflammation process in the submucosal layer of stomachs of male Wistar rats. Therefore PCL microparticles may be considered suitable carriers to reduce the pungency of capsaicinoids mainly after a long-term use. No histological change was verified in the stomachs of the rats that received unloaded-microparticles MC0 (group 3), vehicle (group 4), and saline (group 5).

3.9.2. Obesity induced by hypothalamic lesion using monosodium L-glutamate (MSG)

In order to investigate the anti-obesity effect of capsaicinoids-loaded PCL microparticles, formulation MC5 was evaluated in the experimental animal model of MSG-obesity and compared to control groups.

Fig. 8 compiles the results of Lee index, mesenteric, periepididymal, and retroperitoneal fat pads obtained for MSG-treated (obese) and untreated (control) rats using capsaicinoids (group 1), capsaicinoids-loaded PCL microparticles MC5 (group 2), unloaded-microparticles MC0 (group 3), vehicle (group 4), and saline (group 5). These treatments were performed during 30 consecutive days because each rat month in adulthood is roughly equivalent to 2 human years.

In general, higher values of Lee index and fat deposits were observed for MSG-treated rats compared to control rats which demonstrated an effective induction of obesity using MSG during neonatal period.

Obese rats that received formulation MC5 (MSG-treated group 2) presented a Lee index of 305.7 ± 2.1 $g^{1/3}$/cm. This value was statistically

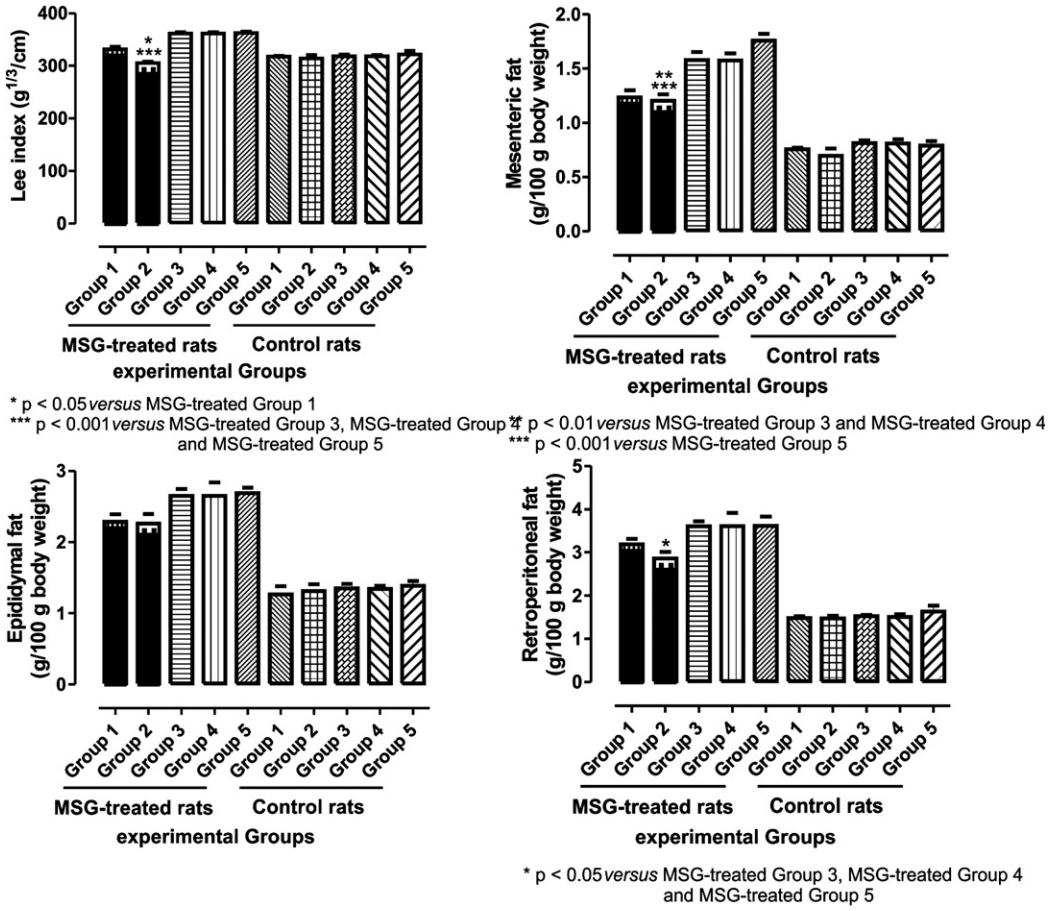

Fig. 8. Lee index, mesenteric, periepididymal, and retroperitoneal fat pads for MSG-treated (obese) and MSG-untreated (control) rats after the 30-day treatment using capsaicinoids (Group 1), capsaicinoids-loaded PCL microparticles MC5 (Group 2), unloaded-microparticles MC0 (Group 3), vehicle (Group 4), and saline (Group 5).

lower than other MSG-treated groups including obese rats that received pure capsaicin (MSG-treated group 1) ($p < 0.05$). The Lee index is a well-established parameter to assess obesity in rats and is equivalent to body mass index (BMI) for humans [32]. Thus formulation MC5 was the most effective strategy for weight loss in obese rats. As expected, the Lee index of MSG-treated group 1 that received capsaicinoids showed a significant difference with other MSG-treated groups: unloaded-microparticles MC0 (MSG-treated group 3), vehicle (MSG-treated group 4), and saline (MSG-treated group 5) ($p < 0.01$).

Regarding the mesenteric fat, MSG-treated group 2 demonstrated the lowest absolute value of fat among animals with central obesity: 1.20 ± 0.06 g/100 g body weight. This result was statistically lower than that verified for MSG-treated group 3 (1.58 ± 0.07 g/100 g, $p < 0.01$), MSG-treated group 4 (1.57 ± 0.07 g/100 g, $p < 0.01$), and MSG-treated group 5 (1.76 ± 0.06 g/100 g, $p < 0.001$). However, no significant difference was observed between MSG-treated group 2 and MSG-treated group 1 (1.24 ± 0.06 g/100 g, $p > 0.05$) which indicates that formulation MC5 has the same potential for reducing mesenteric fat deposits than pure capsaicinoids.

According to the literature [33], visceral adipose tissue (mesenteric fat) contributes to pro-inflammatory factors for cardiovascular disease since it may produce and release a variety of cytokines leading to induce a progressive infiltration of macrophages. Therefore the results obtained for both microparticles MC5 and capsaicinoids show a clinical value because they may be useful for reducing the risk of coronary heart disease in obese patients.

Wistar rats with obesity induced by hypothalamic lesion exhibited values of periepididymal fat of 2.29 ± 0.10, 2.26 ± 0.14, 2.65 ± 0.10, 2.65 ± 0.19, and 2.69 ± 0.09 g/100 g body weight for MSG-treated groups 1, 2, 3, 4, and 5, respectively. Considering periepididymal fat pads, no significant difference was achieved among groups ($p > 0.05$).

Among obese animals, the lower retroperitoneal fat (2.86 ± 0.15 g/100 g body weight) was recorded for MSG-treated group 2. This result was statistically lower than that obtained for MSG-treated group 3 (3.61 ± 0.12 g/100 g), MSG-treated group 4 (3.61 ± 0.31 g/100 g) and MSG-treated group 5 (3.62 ± 0.22 g/100 g) ($p < 0.05$). As previously reported for mesenteric fat, there was no significant difference between MSG-treated group 2 and MSG-treated group 1 (3.19 ± 0.13 g/100 g, $p > 0.05$), which suggests that formulation MC5 has the same potential for decreasing retroperitoneal fat pads than pure capsaicinoids.

In addition, no significant change in Lee index and fat deposits was verified in MSG-untreated (control) rats ($p > 0.05$) during the 30-day treatment. This result may be also considered suitable due to formulation MC5 and capsaicinoids had no negative impact in normal animals as underweight symptoms.

Based on in vivo experimental results, capsaicinoids-loaded PCL microparticles, particularly formulation MC5, represent a viable oral carrier in order to control drug release along gastrointestinal tract and to decrease mesenteric and retroperitoneal fat deposits in obese rats. Concerning the Lee index, formulation MC5 demonstrated improved performance than pure capsaicinoids.

4. Conclusion

In summary, PCL microparticles containing capsaicinoids were successfully obtained by simple emulsion/solvent evaporation. Micrometer-sized formulations with high drug-loading efficiencies were achieved. Additionally, PCL microparticles were able to control the release of capsaicinoids without changing its biexponential release kinetic. The selected formulation MC5 combined an enhanced gastric tolerability with a good pharmacodynamic effect characterized by a reduction of fat deposits in obese rats. In this way, PCL microparticles can be considered as feasible oral carriers for controlled release of capsaicinoids as an innovative strategy of low irritative effect for long-term use in obesity.

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