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ORIGINAL ARTICLE

Preparation and characterization of Δ[9]-tetrahydrocannabinol-loaded biodegradable polymeric microparticles and their antitumoral efficacy on cancer cell lines

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Abstract

Cannabinoids present an interesting therapeutic potential as antiemetics, appetite stimulants in debilitating diseases (cancer, AIDS and multiple sclerosis), analgesics, and in the treatment of multiple sclerosis and cancer, among other conditions. However, despite their high clinical potential, only few dosage forms are available to date.

In this paper, the development of Δ^9-tetrahydrocannabinol (THC) biodegradable microspheres as an alternative delivery system for cannabinoid parenteral administration is proposed. Tetrahydrocannabinol was encapsulated into biodegradable microspheres by the oil-in-water (o/w) emulsion solvent evaporation method. Several formulations were prepared using different drug:polymer ratios. The influence of antioxidant (α-tocopherol acetate) concentration on the release of THC from the microparticles was studied. Elevated process yield and entrapment efficiencies were achieved. The *in vitro* drug release studies showed that the encapsulated drug was released over a two week period. As THC has shown therapeutic potential as anticancer drug, the efficacy of the microspheres was tested on different cancer cell lines. Interestingly, the microspheres were able to inhibit cancer cell proliferation during the nine-day study period. All the above results suggest that the use of biodegradable microspheres would be a suitable alternative delivery system for THC administration.

Keywords

Δ^9-Tetrahydrocannabinol, cancer, cannabinoids, drug delivery, microencapsulation

History

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Introduction

The plant *Cannabis sativa* L. produces over 421 chemical compounds, including about 60 terpeno-phenol molecules named phytocannabinoids which are unique to it [1,2]. Among them, most attention has been paid to Δ^9-tetrahydrocannabinol (Δ^9-THC), which is the most psychotropic component and binds to specific G-protein coupled receptors named cannabinoid (CB_1 and CB_2) receptors [3].

Although cannabis has been used in medicine for millennia, concern over the dangers of abuse led to the banning of its medicinal use in most countries in the 1930s. Only recently, has the therapeutic value of marijuana and individual natural and synthetic cannabinoid receptor agonists and antagonists, as well as chemically related compounds, been reaccepted. Individual cannabinoid receptor agonists made their first entry into the clinic less than 30 years ago. Marinol® (dronabinol) and Cesamet® (nabilone) are oral formulations which are prescribed as anti-emetics for patients receiving chemotherapy, and also as appetite stimulants, for example, for AIDS patients experiencing excessive loss of body weight. In 2005, Sativex®, a sublingual spray which contains approximately equal amounts of Δ^9-THC and the non-psychoactive plant cannabinoid, cannabidiol, received approval for prescription for the symptomatic relief of neuropathic pain in adults with multiple sclerosis and, since August 2007, as an adjunctive analgesic treatment for adult patients with advanced cancer [4]. More recently, Sativex® has also been approved for the treatment of spasticity in multiple sclerosis in several European countries.

CB_1/CB_2 receptor agonists also have a number of other potential therapeutic applications. These include the relief of pain induced by certain disorders or conditions in addition to cancer and multiple sclerosis; inhibition of angiogenesis and growth of malignant tumors; relief from various symptoms of multiple sclerosis, spinal cord injury, Alzheimer's disease and amyotrophic lateral sclerosis; relief from tics and behavioral problems experienced by patients with Tourette's syndrome; and management of glaucoma, to name just a few [4–8].

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Despite the promising clinical potential of cannabinoids, only the three pharmaceutical preparations described above are commercially available.

Moreover, although the two main modes of cannabinoid administration are smoking of dry cannabis plant material and oral ingestion of dronabinol capsules (Marinol®), they have specific disadvantages which prompt the development of more reliable administration forms [9–15]. Disadvantages of smoking include variable drug content, mucosal damage, inhalation of carcinogens formed by pyrolysis and short duration of effect, whereas disadvantages of oral ingestion include cannabinoid instability and acidic gastric pH [16], slow and erratic absorption, delaying onset of action and low systemic bioavailability [6,17].

In addition, it should also be taken into consideration that THC's lipophilicity (solubility in water 2.8 mg/mL, log *P* 3.78) [18,19], viscous tar-like nature and instability (Δ^9-THC is susceptible to decomposition by oxidation, heat, acid and light) [20,21] restrict its use in most pharmaceutical preparations and some research experiments [14,22,23].

Although in recent years several alternative modes of cannabinoid delivery have been investigated, such as smokeless oral inhalers (aerosols), transdermal patches and rectal suppositories, the need for suitable cannabinoid formulations persists [24].

Therefore, the aim of our research work has been to develop alternative formulations for cannabinoid administration. The results of our studies with cannabidiol have been recently published [25]. The aim of the present study was to transform viscous, resin-tar like THC into free flowing microspheres which could be easily handled and weighted. This manuscript describes the development and *in vitro* characterization of the formulations. Since in recent years an impressive antitumoral efficacy of THC has been described [8,26–31], the long-term *in vitro* antitumoral properties of the developed THC microparticles (MP) were evaluated in different cancer cell lines. These MP are intended for a sustained release of THC after their peritumoral subcutaneous administration, reducing dosing frequency.

Materials and methods

Materials

Δ^9-Tetrahydrocannabinol (THC) was kindly provided by THC Pharm (Frankfurt, Germany). THC is a highly lipid-soluble viscous compound with a very high octanol-water partition coefficient, reported to be of the order of 6000 [32,33].

Polycaprolactone (PCL) was chosen as the polymer to prepare the microparticles described in this work because, unlike polymers and copolymers based on lactides (PLA) and glycolides (PGA), PCL degradation does not generate and acidic environment which could adversely affect the stability of the encapsulated cannabinoid [34,35].

Poly-ε-caprolactone (PCL) (Mw 42 500), Sigmacote, polyvinyl alcohol (PVA) and α-tocopherol acetate were purchased from Sigma Aldrich (St Louis, MO). Methylene chloride (DCM), potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate dihydrate ($Na_2HPO_4{\cdot}2H_20$) and Tween®-80 were obtained from Panreac (Barcelona, Spain).

Methanol (HPLC-grade) and acetonitrile (HPLC-grade) were purchased from LabScan (Dublin, Ireland). Double-distilled water was used throughout. All chemicals were used without further purification.

Methods

Preparation of microspheres

The o/w emulsion solvent evaporation methodology already developed for cannabidiol microencapsulation [25], was used as a starting point for the preparation of THC-loaded MP. Briefly, THC and PCL were dissolved in 5 mL of methylene chloride to form the organic solution. Then, this solution was slowly added into 250 mL of an aqueous solution containing 0.5% of PVA and emulsified at 3000 rpm for 6 min to form a stable o/w emulsion. Subsequently, solvent removal and hardening of the microspheres was achieved by continuous stirring at 200 rpm for up to 3 h. Finally, the MP were isolated by filtration and washed with distilled water several times to remove PVA. The MP thus produced were freeze-dried for 12 h at −60°C, 200 mT and stored at −20 °C.

Different formulations were prepared from several initial drug to polymer ratios, namely 15/150, 30/150 and 50/150. Also, an antioxidant (α-tocopherol acetate) was added at different proportions to the organic phase (0.05 or 5%) in order to prevent THC oxidation.

Placebo microspheres were prepared in a similar manner except that THC was not incorporated.

Morphological characterization

The surface topography of the MP was studied by scanning electron microscopy (SEM) (Jeol JSM-6400, Tokyo, Japan). MP samples were mounted onto stubs using double-sided adhesive tape. The stubs were then vacuum coated with gold using fine coat ion sputter (Emitech K550X, Emitech Ltd., UK).

Particle size measurements

The mean diameter and particle size distribution were examined by dynamic laser light scattering using a particle size analyser (Leeds & Northrup Instruments, Ireland).

The MP were first suspended in distilled water by sonication for 2 min. Then, the dispersion was added to the sample dispersion unit containing a stirrer.

Δ^9-tetrahydrocannabinol content and encapsulation efficiency

To determine the Δ^9-THC content, 10 mg of MP were dissolved in 1 mL of DCM and then 9 mL of HPLC mobile phase were added into this solution to extract the THC. The above suspension was vigorously mixed by vortexing, and filtered through 0.45 mm PTFE-filters (Millipore) to remove the polymeric debris. The clear solution was analyzed for Δ^9-THC content by high-performance liquid chromatography. The analytical conditions were as follows: column: Mediterranea®-Sea C-18 (5 μm; 150 × 4.6 mm i.d., Teknokroma, Barcelona, Spain); mobile phase: acetonitrile:water (85:15 v/v); flow rate: 1 mL/min; injection volume: 20 μL; detection: 205 nm.

The % drug loading and % encapsulation efficiency of the MP were calculated as:

$$\%\ \text{Drug loading} = \frac{\text{Weight of drug in MP}}{\text{Weight of MP}} \times 100 \quad (1)$$

$$\%\ \text{Encapsulation efficiency (EE)} = \frac{\%\ \text{Drug loading}}{\%\ \text{Theoretical loading}} \times 100 \quad (2)$$

Differential scanning calorimetric studies

Thermal analysis were carried out to investigate the physical state of the drug within the MP and to evaluate the effect of drug loading onto polymer crystallinity.

The area of the melting peak is related to the heat of fusion and degree of crystallinity of the sample. By studying the location and width of the melting peak, the crystallinity of the sample can be examined. The melting points are taken to be the onset of melting as is the convention in differential scanning calorimetric (DSC).

The heat of fusion (ΔH) reported for PCL is the value measured normalized by the percent PCL of in the sample. The percent crystallinity of the PCL phase is determined by:

$$\lambda_m = \frac{\Delta H}{\Delta H_U} \quad (3)$$

where ΔH_U is the enthalpy of fusion for a perfect crystal and is 142.9 J/g for PCL [36].

DSC scans were performed using a Mettler–Toledo differential scanning calorimeter DSC820 with a Huber TC100 intracooler and nitrogen purge gas. Samples weighing 3-5 mg were sealed in aluminium sample pans. An empty sealed aluminium pan was used as reference. The heating rate was 10 °C/min in a nitrogen atmosphere (flow rate: 10 mL/min).

In vitro *drug release studies*

Samples of MP weighing 15 mg were suspended in 30 mL PBS pH 7.4 containing 0.1% Tween-80 (to maintain *sink* conditions) and placed in glass vials. These vials were incubated in a water shaker bath (Clifton® NE 5-28, UK) at 37°C with constant agitation (100 strokes/min). Aliquots of the dissolution medium (28 mL) were withdrawn at predetermined time intervals using a syringe fitted with a 25-gauge needle (to avoid MP retrieval). The withdrawn volume was replenished with an equal volume of fresh and pre-warmed buffer at 37°C. Samples were filtered through 0.45 μm filters and analyzed by HPLC.

All the experiments were repeated thrice, and the average values were taken.

In vitro *antitumoral activity*

As previous studies have shown that Δ^9-THC exhibits antitumor effects on various cancer cell types [5,37], the efficacy of the Δ^9-THC-loaded MP was tested on RBL2H3 rat basophilic leukaemia cells, and Caco2 human colorectal carcinoma cells.

Cell cultures. RBL2H3 cells were grown in EMEM (Biowhitaker®, Lonza, Velviers, Belgium) supplemented with 10% FBS, 1% penicillin-streptomycin, L-glutamine (200 mM) at 37 °C in an humidified incubator containing 5% CO_2. Caco2 cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L of glucose and L-glutamine (Biowhitaker®, Lonza, Velviers, Belgium).

Cells in an exponentially growing phase were used for the cytotoxicity experiments.

Cell proliferation assay. Cells were seeded at a density of 50 000 cells/well on six-well culture plates. Three hours after seeding, treatment was added to the medium. The Δ^9-THC solution was prepared from an ethanolic stock solution which was further diluted with cell culture medium. In all cases the final ethanol concentration was below 0.001%.

In order to avoid MP removal when changing the cell culture medium, MP were added on top of a 3 μm pore size insert placed on top of each well. While MP were added only at day 0, Δ^9-THC in solution was added daily. Medium was changed every two days.

Cell viability evaluation. The effect on cancer cell viability of free Δ^9-THC and drug-loaded MP was evaluated using the methylthiazolyldiphenyl tetrazolium bromide (MTT) assay at different time points. The assay is dependent on the cellular reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. Briefly, the day of the analysis MTT (5 mg/mL) was added to each well and the cells were incubated at 37 °C for 3 h. Then, blue formazan crystals were dissolved by adding lysis buffer pH 4.7 (which contains 20% SDS and 45% DMF). After a 6 h incubation at 37 °C the optical densities were measured spectrophotometrically (PerkinElmer Lambda 12, Boston, MA) at a wavelength of 630 nm.

A preliminary study (data not shown) demonstrated the lack of cytotoxic effect of the placebo MP. Therefore, the *in vitro* cell viability results presented in the article are expressed as the percentage of cell viability in relation to vehicle or placebo MP-treated wells.

The growth inhibitory rate was calculated by the following formula:

Growth inhibitory rate = (average OD value in the control group-average OD value in the treatment group)/average OD value in the control group × 100%.

Statistical analysis

Statistical analyses were performed using Statgraphics® (Statpoint Technologies, Inc., Warrenton, VA). All the assays were performed in triplicate, and the results were expressed as mean ± S.D.

The *in vitro* release data were fitted to different mathematical models by linear regression analysis. Coefficients of correlation (r^2) were used to evaluate the accuracy of the fit.

Cell viability results of different formulations were compared by ANOVA. When the differences in the means were significant, a Duncan's multiple range test was conducted. Differences in p values below 0.05 were considered statistically significant.

Results and discussion

The o/w emulsion solvent evaporation technique is one of the most commonly used methods for microencapsulation of drugs. These MP are generally smooth, spherical and provide sustained drug release. However, the properties of these MP are greatly influenced by the physicochemical characteristics of the drug to be encapsulated, the polymer, the organic solvent used in their preparation, and the emulsification process.

Morphology and particle size

Scanning electron micrographs of placebo and THC-loaded PCL MP are shown in Figure 1.

In all cases the MP prepared were smooth, spherical, non-porous, well-separated, with good flowability and easy to resuspend in aqueous media. However, those prepared from a 50/150 drug-to-polymer ratio (i–j) appeared in groups. This agglomeration was attributed to the viscous and sticky tar-like nature of Δ^9-THC as well as to its bioadhesive properties [38].

The results of the particle size analysis are listed in Table 1. The particle sizes were not significantly different from one formulation to the other (except for the 50/150 formulation, because of the agglomeration of the particles). The mean MP diameter was between 49 and 60 μm, which is suitable for their subcutaneous administration.

Drug loading and encapsulation efficiency

The results of process yield, drug loading, encapsulation efficiency (EE) and particle size analysis of the MP are presented in Table 1.

Yield was reasonably good in all batches, except of those prepared with the 50/150 proportion.

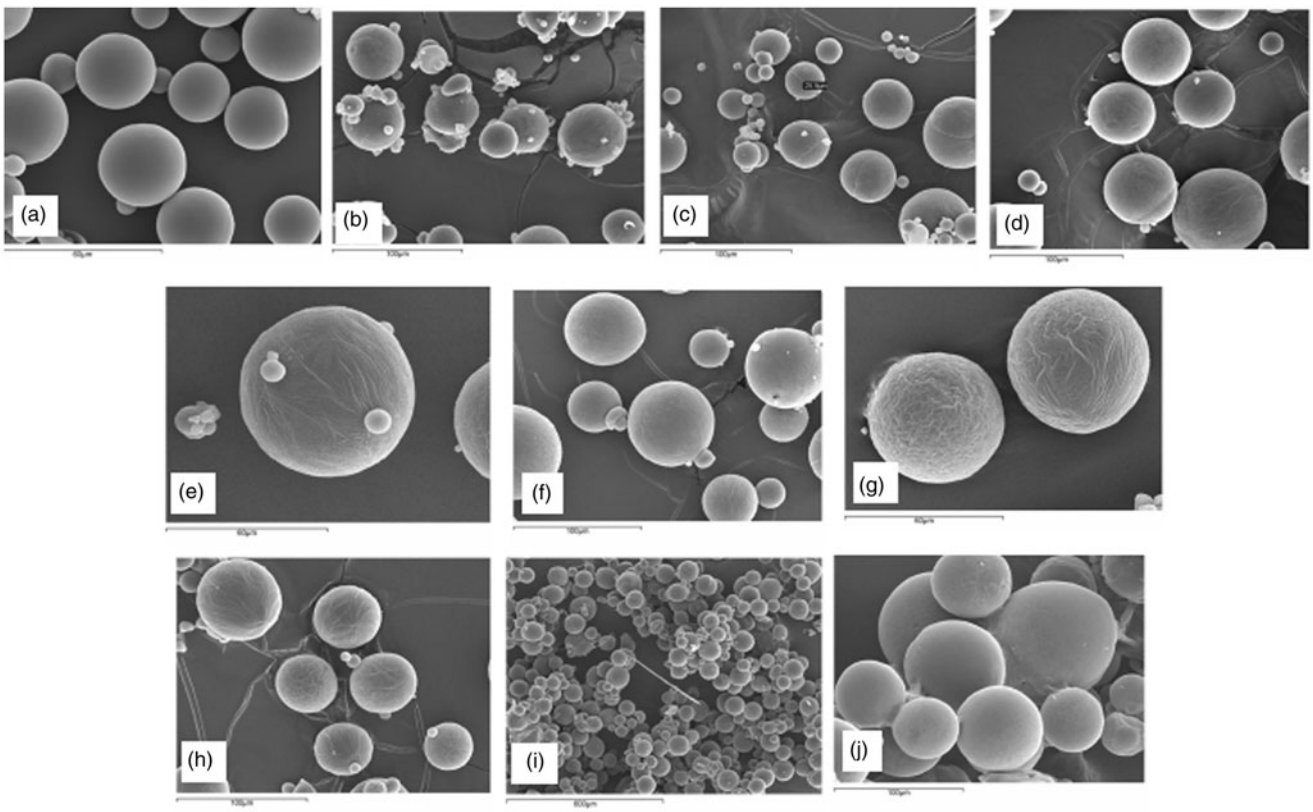

Figure 1. Representative SEM pictures of placebo microspheres (a); THC-PCL MP: 15/150 (b, c), 15/150-0,05% vit E (d), 15/150-5% vit E (e), 30/150 (f), 30/150-0,05% vit E (g), 30/150-5% vit E (h), 50/150 (i, j).

Table 1. Particle size, yield, drug content and encapsulation efficiency of the different formulations ($n = 10$).

Formulation	Mean particle size (μm)	Yield (%)	mg THC/100 mg MP	*EE (%)*
Placebo	36.78	86.18 ± 4.04	–	–
15/150	49.18 ± 21.54	87.34 ± 7.29	9.52 ± 0.45	103.78 ± 5.50
15/150-0.05% vit E	54.34 ± 24.29	75.24	9.084	108.4
15/150-5% vit E	44.64 ± 20.30	81.48 ± 6.20	8.32 ± 1.90	84.55 ± 13.6
30/150	59.46 ± 24.28	70.57 ± 4.03	18.43 ± 3.94	128.32 ± 11.88
30/150-0.05% vit E	51.77 ± 23.48	69.04	15.93	113.39
30/150-5% vit E	60.11 ± 24.84	72.34 ± 2.78	17.82 ± 3.92	119.88 ± 12.92
50/150	87.88 ± 27.63	37.42 ± 2.21	32.69 ± 2.4	97.83 ± 6.21

Microencapsulation of poorly water-soluble drugs like Δ^9-THC by the o/w emulsion solvent evaporation technique results in high entrapment efficiencies because of the low solubility of drug in the outer aqueous phase. For this reason, this technique was selected to prepare the polymeric systems. In fact, as shown in Table 1, very high encapsulation efficiencies were obtained. Values above 100% were attributed to a higher loss of polymer than THC during preparation.

In order to increase the drug loading of the MPs and to minimize the total volume of MPs to be administered, several formulations were prepared using different drug/polymer ratios. Interestingly, as the initial drug-to-polymer ratio increased, the microspheres drug loading increased proportionally, resulting in encapsulation efficiencies close to 100% in all cases. However, as depicted in Figure 1 (i, j) when the ratio 50/150 was employed no free-flowing particles were obtained. Therefore, it was concluded that 30/150 was the highest ratio which could be used to efficiently prepare the MP.

Thermal analysis

The thermal behavior of the developed MP was studied using DSC. The melting behavior of raw PCL and Δ^9-THC-loaded MP was compared. Since the thermograms of the MP prepared with antioxidant (0.05% or 5%) were similar to those obtained from the equivalent formulation without antioxidant, Figure 2 only includes the thermograms of antioxidant-free formulations.

THC raw material showed a Tg at 9.33 °C (thermogram not shown). As depicted in Figure 2, as drug loading increased polymer melting point broadened and shifted to lower temperatures (from 62 °C to 54 °C), suggesting that PCL and Δ^9-THC were miscible. These changes on polymer melting peak when adding Δ^9-THC reflected the plasticizing effect of the drug, which resulted in a modification of the polymer mechanical properties. These changes, together with the adhesive nature of Δ^9-THC [38] may explain why as drug content increased the MP tended to agglomerate, as observed by SEM (Figure 1). Furthermore, these changes on the polymer mechanical properties were observed by a drop of polymer crystallinity (Table 2). In addition, as no changes on polymer cristallinity were observed when preparing the placebo microparticles, it was concluded that the decrease of polymer crystallinity was not due to the microencapsulation process itself, but to the incorporation of the drug.

In vitro release studies

Figure 3 shows the release profiles of THC from different formulations of MP depending on the initial drug/polymer ratio and the antioxidant concentration.

The variability in drug loadings did not have a significant influence on Δ^9-THC release from the PCL-microcarrier samples (Figure 3A), which generally showed a biphasic

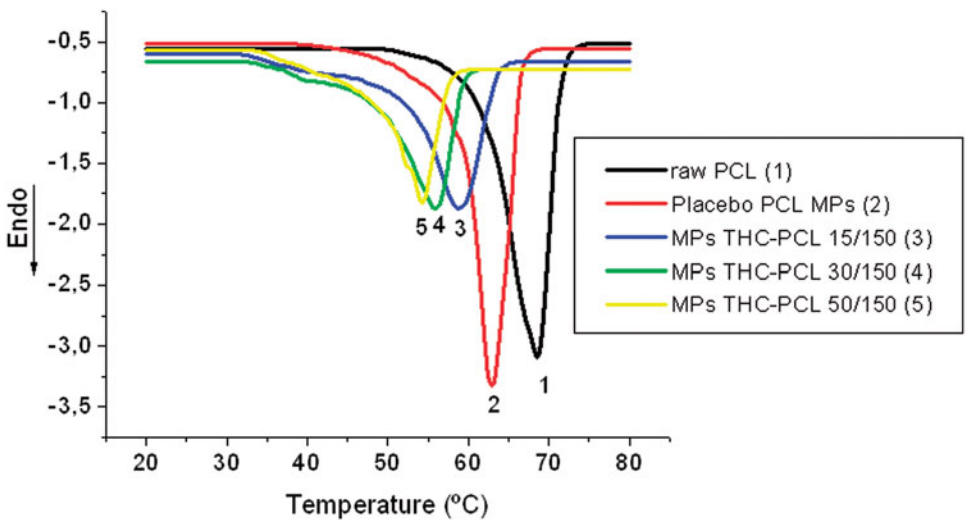

Figure 2. DSC thermograms of PCL raw material, placebo MP and 15/150, 30/150 and 50/150 THC-loaded MP ($n = 3$).

Table 2. Melting temperature, enthalpy and crystallinity of PCL in different formulations studied by DSC.

Sample	*T*m (°C)	λH_m^* (J/g)	λ_m (%)
Raw PCL	66.95 ± 1.24	102.09 ± 2.36	73.69 ± 2.56
Placebo MPs	62.28 ± 0.38	98.84 ± 4.17	70.85 ± 2.99
MPs THC-PCL 15/150	58.44	77.62	52.65
MPs THC-PCL 15/150-0,05% vit E	57.82	70.84	50.78
MPs THC-PCL 15/150-5% vit E	57.44	59.25	50.23
MPs THC-PCL 30/150	55.70	71.99	52.61
MPs THC-PCL 30/150-0,05% vit E	55.48	73.78	52.90
MPs THC-PCL 30/150-5% vit E	54.80	73.48	52.68
MPs THC-PCL 50/150	54.01	68.05	48.78

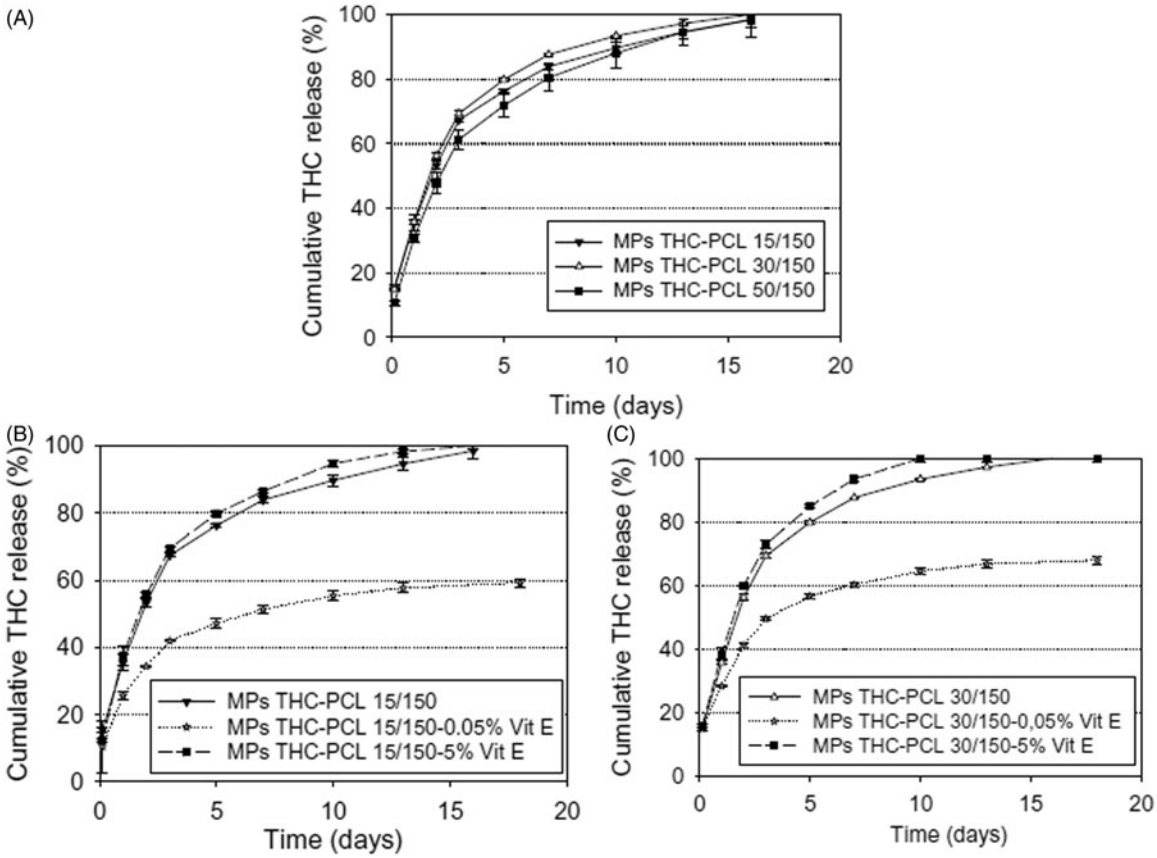

Figure 3. *In vitro* drug release profiles of the different formulations of THC-PCL MP (A) formulations prepared without antioxidant; (B) 15/150 MPs prepared with or without vitamin E acetate (0.05 and 5%); (C) 30/150 MPs prepared with or without vitamin E acetate (0.05 and 5%). Data are represented as mean ± SD ($n = 3$).

Table 3. Influence of vitamin E addition on Δ^9-THC release from the MP.

Formulation	·	f_2	Conclusion
MP THC-PCL 15/150 0.05% vitE	36.75	30.53	Not similar
MP THC-PCL 15/150 5% vitE	4.93	74.19	Similar (2–5% difference)
MP THC-PCL 30/150 0.05% vitE	27.07	37.08	Not similar
MP THC-PCL 30/150 5% vitE	6.33	69.45	Similar (difference < 5%)

release behavior. The experimental results were fitted to Higuchi, first-order and Korsmeyer–Peppas kinetic models.

No differences between Higuchi and first-order kinetics adjustments were shown, but the best r values corresponded to the Korsmeyer–Peppas model. The n values in this model were close to 0.5 in all cases (range 0.35–0.54) suggesting that the mechanism involved in the Δ^9-THC release from the matrices was drug diffusion. Indeed, due to the semicrystalline nature of PCL, water can penetrate easily into the amorphous part of the polymer facilitating the release of drug by diffusion.

The influence of antioxidant concentration onto the Δ^9-THC release from the MP was also evaluated (Figure 3B–C). The comparison of dissolution profiles was performed using a model independent method [39]. The results are presented in Table 3. The formulations with 5% vitamin E acetate exhibited a similar release profile as the antioxidant-free MP ($f_2 = 74.19$ and 69.45), while the release rate of the drug was lowered in the presence of 0.05% vitamin E ($f_2 = 30.53$ and 37.08). In fact, when 0.05% antioxidant was added, the maximum release was around 60%. This ability of vitamin E to modulate *in vitro* drug release from MP has been previously observed; however, it seems to be dependent on the polymer, the drug and the antioxidant concentration. Barcia and colleagues [40] have reported that fatty additives such as tocopherol slowed the release of ganciclovir from PLGA MP whereas Martínez-Sancho and colleagues [41] found the opposite effect after 14 days when MP of the same polymer were loaded with aciclovir and 5% α-tocopherol. Mu and Feng [42] reported a lower paclitaxel release from PLGA nanoparticles after the addition of a tocopherol derivative (vit E TPGS) to the polymeric matrix. As explained by the authors this effect might be related to the interaction or affinity between the hydrophobic drug and the polymeric matrix. Forrest and colleagues [43] incorporated the highly lipophilic α-tocopherol (log $P_{\text{octanol/water}}$ 9.96) in rapamycin loaded PEG-PCL micelles. Powder X-ray diffractometry demonstrated that the addition of α-tocopherol did not significantly affect the crystallinity of the PCL blocks. However, the melting points and core viscosity of the micelles decreased with increasing amounts of α-tocopherol (15%–35%). The authors indicate that a possible explanation for this discrepancy is α-tocopherol may form separate phases, or pockets,

within the micelle core, that would still allow an overall crystalline PCL core. When rapamycin release was investigated, the incorporation of α-tocopherol slowed down its release rate. Drug association and interaction with the MP core can influence the drug's release rate, so the effect on drug release of core characteristics modified by the addition of α-tocopherol were investigated.

Because incorporation of α-tocopherol did not change the encapsulation of Δ^9-THC in PCL MP (Table 1), it was postulated that α-tocopherol would not have an influence on the THC release rate as seen for the 5% concentration. However, although a low α-tocopherol concentration (0.05%) did not change the encapsulation percentage of THC in PCL MP, it slowed down its release rate. The diffusion rate of drug from the core is dependent on factors including crystallinity, viscosity and drug association. DSC studies showed no significant changes neither on the crystallinity of the polymeric matrix nor on its melting temperatures. Therefore, the restriction to drug release at low α-tocopherol concentrations could be related to a higher hydrophobicity of the MP core due to an interaction between the antioxidant and PCL, whereas at the higher antioxidant concentration it might form microseparate phases within the polymer that would not affect drug diffusion through the MP matrix. PCL is a semi-crystalline polymer with a *T*g around −60 °C. This temperature zone was not included in our DSC studies, therefore the lack of evidence for antioxidant polymer interactions suggest these might occur within the amorphous domains of the polymer.

Since the formulations prepared with 0.05% antioxidant did not show a complete drug release, only those MP prepared with 5% antioxidant were selected for further studies.

Cell cultures

Cell culture studies are a commonly used approach to test whether the biological activity of drugs incorporated in microspheres is preserved after encapsulation. *In vivo* drug concentration is time and space dependent whereas dose–response cell culture experiments are conducted by incubating cells in a spatially uniform environment with a fixed extracellular drug concentration. Therefore, an extrapolation of *in vitro* release data to the *in vitro* cell culture situation and even these later studies to the *in vivo* situation is difficult and it depends on the therapeutic approach selected. In order to mimic the *in vivo* situation as much as possible, and avoid MPs suction when changing the cell culture medium, MP were added on top of 3 μm pore size inserts which were placed on top of each well. Incubation media was replaced every two days to simulate the clearance of drug taking place in the tumor because of the leaky microvasculature.

For the cancer cell viability tests, the 15/150 and 30/150 MP were selected. The effects of the THC-loaded MP and free THC (positive control) are shown in Figure 4. When placebo PCL MP were tested no cytotoxicity was observed (data not shown). As depicted in Figure 4, Δ^9-THC loaded MP inhibited cell growth of both cancer cell lines during the 9-days of exposure due to the sustained release properties shown in Figure 3. No differences on cell growth inhibition were observed between the formulations prepared without or with 5% vitamin E acetate. The exposure time could not be extended due to the limitations of the technique, i.e. the cells on control wells were reaching confluence and, hence, would be detaching and dying thereafter. The obtained results confirm that the Δ^9-THC released from the MP maintained its

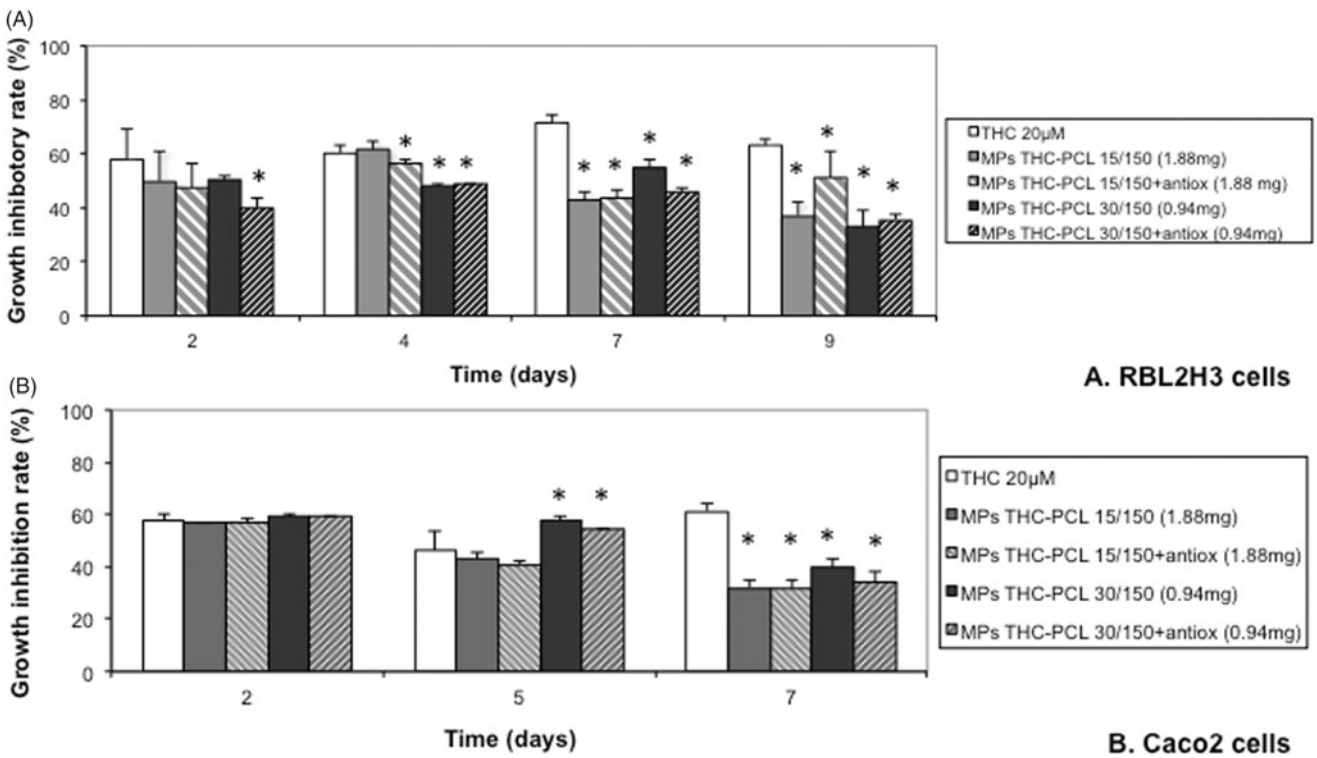

Figure 4. Antitumoral effect of free THC and THC-loaded MP on RBL2H3 (A) and Caco2 (B) cells. An asterisk represents a statistically significant difference between ''free'' Δ^9-THC and Δ^9-THC-loaded MP ($p < 0.05$).

biological activity. However, after 7 days of incubation, the effect of the MP formulations which, unlike the ''free'' Δ^9-THC, were administered only at the beginning of the assay, started to decrease, in agreement with the results from the *in vitro* release studies (Figure 3).

In fact, when THC was added in solution, a constant drug concentration of 20 μM was maintained all over the incubation period. In contrast, when Δ^9-THC loaded MP were incorporated into the insert, Δ^9-THC was slowly released to the medium. According to the *in vitro* release studies, 50% of the drug would be released within the first two days, a total of 84 μg THC that would approximately represent a total cell exposure of 88 μM × day according to the area under the concentration-time curve as compared to 40 μM × day in the solution. Later on, the culture medium was replaced and 34 μg of THC would be additionally released in the following 2 days. Assuming the first-order drug release kinetics drug exposure would be 36 μM × day between days 2 and 4 of incubation. The next interval amounts a cell exposure of 42 μM × day and finally, from day 7 to day 9 of incubation the exposure was lower (9 μM × day). Indeed, Δ^9-THC released from the MP shows greater inhibitory activity at day 4, and delayed with regards to maximal exposure suggesting some kind of a lag phase mechanism. According to the *in vitro* drug release results, the total drug exposure was comparable in both cases, being 175 μM × day with THC-MP with or without 5% vitamin E acetate versus 180 μM × day with THC solution. However, different cell viability results were obtained. This may be due to a different THC release from MP in the cell culture experiment with respect to the drug release studies. In other studies using MP, a significant reduction of the *in vitro* release rates were observed *in vivo* [44].

With regards to Caco2 cells, after the first two days the inhibitory effect with THC in MP was comparable to that achieved with the drug in solution. As shown in Figure 4(B), a general trend towards less inhibitory effect with incubation time was observed, in accordance to the progressive decline in THC release.

Conclusions

Despite the promising pharmacological potential of cannabinoids, their high lipophilicity restricts their use in most pharmaceutical preparations and some research experiments. In fact, Δ^9-THC is an oily viscous resin that is very sticky at ambient conditions making it difficult to handle and to accurately weight small amounts and prepare suspensions for injection. In the present work, Δ^9-THC was efficiently encapsulated into PCL MP. This represents the first formulation developed to exploit the antitumoral properties of this compound. The obtained free-flowing MP were spherical in shape and had an average size under 100 μm, enabling their subcutaneous administration through a conventional needle. High process yields and encapsulation efficiencies were obtained. The obtained MP showed a sustained release of Δ^9-THC over a 2-week period. Furthermore, *in vitro* cancer cell viability studies revealed that the encapsulated drug maintained its antitumoral properties. Moreover, it was demonstrated that a single administration of the MP showed an antiproliferative effect during the 9 days of the experiment. These results suggest that polymeric MP could be considered as an alternative delivery system for Δ^9-THC administration, not only for pharmacological studies but also for a future clinical application. Moreover, when long-term treatments are intended, for example, as antitumoral drugs, these systems would reduce dosing frequency and enhance patient compliance. Ongoing *in vivo* studies will further establish the antitumoral potential of the developed MP. In the future, these systems might be used in combination with other anticancer drugs or conventional therapies in order to increase therapeutic efficacy.

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Declaration of interest

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

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