



# Development, Characterization and *In Vitro* Gastrointestinal Release of PLGA Nanoparticles Loaded with Full-Spectrum Cannabis Extracts

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## Abstract

Cannabinoids, such as  $\Delta^9$ -tetrahydrocannabinol (THC) and cannabidiol (CBD), are effective bioactive compounds that improve the quality of life of patients with certain chronic conditions. The copolymer poly(lactic-co-glycolic acid) (PLGA) has been used to encapsulate such compounds separately, providing pharmaceutical grade edible products with unique features. In this work, a variety of PLGA based nanoformulations that maintain the natural cannabinoid profile found in the plant (known as full-spectrum) are proposed and evaluated. Three different cannabis sources were used, representing the three most relevant cannabis chemotypes. PLGA nanocapsules loaded with different amounts of cannabinoids were prepared by nanoemulsion, and were then functionalized with three of the most common coating polymers: pectin, alginate and chitosan. In order to evaluate the suitability of the proposed formulations, all the synthesized nanocapsules were characterized, and their cannabinoid content, size, zeta-potential, morphology and *in vitro* bioaccessibility was determined. Regardless of the employed cannabis source, its load and the functionalization, high cannabinoid content PLGA nanocapsules with suitable particle size and zeta-potential were obtained. Study of nanocapsules' morphology and *in vitro* release assays in gastrointestinal media suggested that high cannabis source load may compromise the structure of nanocapsules and their release properties, and hence, the use of lower content of cannabis source is recommended.

**Keywords** cannabinoids · full-spectrum · PLGA · nanoencapsulation

## Abbreviations

CI Chemotype I  
CII Chemotype II

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CIII	Chemotype III;
CBC	Cannabichromene
CBCA	Cannabichromenic acid
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBDV	Cannabidivarin
CBDVA	Cannabidivarinic acid
CBG	Cannabigerol
CBGA	Cannabigerolic acid
CBN	Cannabinol
CBNA	Cannabinolic acid
DAD	Diode-array detector
EE %	Encapsulation-efficiency
HPLC	High-performance liquid chromatography
IS	Internal standard
$K_H$	Higuchi's model constant
$r^2$	Coefficient of determination
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
THC	$\Delta^9$ -Tetrahydrocannabinol
THCA	$\Delta^9$ -Tetrahydrocannabinolic acid



THCV	$\Delta^9$ -Tetrahydrocannabivarin
THCVA	$\Delta^9$ -Tetrahydrocannabivarinic acid
PDI	Polydispersity index
PLGA	Poly(lactic-co-glycolic acid)
PVA	Polyvinyl alcohol

## Introduction

The therapeutic strength of *Cannabis Sativa L.* has been well-known in various cultures from 3000 BC to the present days [1]. The medicinal properties of the plant comes from the various bioactive compounds that have their own palm, such as, terpenes, flavonoids, alkaloids and, most importantly, cannabinoids, which are specific of the Cannabis plant [1, 2]. Cannabinoids interfere in the endocannabinoid system, favoring a variety of psychological and physiological effects due to the different affinities between cannabinoids and endocannabinoid system's receptors (mainly CB<sub>1</sub> and CB<sub>2</sub>) [2, 3].

Among the various cannabinoids (more than 120) identified in the literature,  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC or THC) and cannabidiol (CBD) still are the most known and widely used ones, since they are the major cannabinoids in most cannabis plants and they have shown to be effective to relief the symptoms of chronic pain and other diseases [4]. THC is widely known for its psychoactivity, but also for its analgesic, anti-inflammatory, appetite stimulant and antiemetic properties [5]. CBD, has shown also analgesic and anti-inflammatory effects, together to anxiolytic, anti-epilepsy and antipsychotic effects [6]. Because they have such diverse effects, the choice between THC or CBD is made depending on the therapeutic application, although the combination of both has proven to be practical in reducing the side effects of THC (i.e., toxic psychosis, dizziness, dry mouth...), so the combination of both is often sought [7]. Besides THC and CBD, minor cannabinoids such as cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN) and tetrahydrocannabivarin (THCV), have demonstrated therapeutic efficacy for different applications, although the main interest towards these cannabinoids is their use as enhancers of the effects of major cannabinoids [8]. As with the combination of THC and CBD, the simultaneous use of multiple cannabinoids can improve the therapeutic benefits of major cannabinoid. Indeed, it is believed that consuming the full spectrum of cannabinoids present in the plant may promote a synergistic effect or "entourage" effect of major cannabinoids. For this reason cannabis-derived products that maintain the cannabinoid composition of the plant (usually referred to as full-spectrum products) have gained interest in the recent years [5, 8, 9]. Related to this, the extended knowledge in the plant, including genetics and cultivation conditions, has led to the option to develop a vast variety of

Cannabis varieties, each of them having a unique spectrum of cannabinoids [10].

Nowadays, a wide variety of cannabis-derived products are available ranging from vaporizable plant extracts to creams and lotions. Nevertheless, when it comes to medicinal use, products designed for oral administration are preferred because of their ease of dosing and self-medication, their reduced toxicity and the ability of providing long-lasting effects [7, 11, 12]. The mayor drawback faced by edible products is the low oral bioavailability of cannabinoids. This is partly due to the lipophilic nature of cannabinoids (log P ~ 6–7), which makes them to have a poor solubility in aqueous media (2–10  $\mu\text{g/mL}$ ) and negatively affects their absorption in the intestine [13, 14]. Additionally, cannabinoids have low stability in acidic and oxidative environments, especially in the presence of light and heat [15, 16]. Therefore, cannabinoids can easily be degraded through the digestive tract, particularly in the stomach [17]. The matrix in which cannabinoids are found can play a crucial role in improving their solubility and physicochemical stability, and hence, their pharmacokinetics and bioavailability [18]. Encapsulation of cannabinoids in polymeric vehicles has proved to be a suitable strategy for trapping cannabinoids in matrixes that provide enhanced oral bioavailability, while offering the possibility to design formulations with specific release profiles [18–20]. In this regard, polymeric nanoformulations have emerged as a promising tool to provide a targeted delivery of cannabinoids, promote improved oral bioavailability and desired pharmacokinetic profiles [20–23].

Within the wide range of polymeric materials that can be used to elaborate nanocarriers, poly(lactic-co-glycolic acid) (PLGA) can be highlighted due to its: (i) biodegradability and biocompatibility, (ii) ability to provide a sustained, controlled and targeted release, and, (iii) versatility in terms of routes of administration (e.g., oral, topical, ocular, transdermal) among other properties [24–27]. Those properties have led to PLGA as one of the most used polymeric material to design pharmaceuticals containing a wide variety of bioactive compounds [25, 27]. For instance, PLGA-based nanoformulations have recently been proposed to encapsulate various drugs and bioactive compounds, such as coumarin C75, oxaliplatin, *Leishmania infantum* antigens and quercetin. The use of PLGA nanocarriers has improved solubility and cellular uptake of such compounds, and in general, increased the efficacy of their pharmacological action [28–31].

In the field of cannabinoids, Fraguas-Sánchez and coworkers, developed CBD-loaded PLGA injectable nanoparticles for the treatment of different types of cancer (ovarian and breast) with very promising results [32, 33]. Likewise, the research group of Martin-Banderas has developed a series of PLGA nanoformulations loaded with CB13 (a synthetic cannabinoid derivative) designed

for oral administration. These formulations have shown to offer an improved intestinal absorption and biodistribution of cannabinoids, sustained long-term (10–14 days) release of cannabinoids, and minimal cytotoxicity, making them promising formulations for treating chronic pain, among other potential uses [34–37].

Nonetheless, it has been demonstrated that the addition of an outer layer (or coating) by the functionalization of PLGA nanocapsules, using different polymers can even further improve their characteristics in terms of absorption, biodistribution and therapeutic efficacy [30, 34–39]. For instance, chitosan is a natural cationic polysaccharide that has been widely used to functionalize PLGA nanoparticles. The inclusion of a chitosan coating can significantly improve intestinal permeability and absorption of PLGA nanoparticles, as it is able to open tight junctions in the intestinal epithelium [40–44]. Despite the proven efficacy of chitosan, other well-known natural polysaccharides, such as alginate and pectin, can also be used to improve the features of PLGA nanoparticles [45]. Pectin and alginate are anionic polymers, unlike chitosan, are more stable at low pH values and are therefore used for intestinal and colonic drug delivery [44]. Pectin is commonly used to enhance shelf-life of encapsulated bioactive compounds, prevents irritation of the intestinal mucosa, and can improve intestinal retention, intestinal mucoadhesivity and transport properties of capsules [44, 46–48]. Similarly, alginate is a common coating that can improve encapsulation efficiency, integrity of capsules through the gastrointestinal track, and their mucoadhesivity, so it has been used previously to improve release and absorption properties of PLGA nanoformulations [38, 49–51].

In this context, and highlighting the fact that the cannabinoid-PLGA formulations developed to date only contain THC, CBD or CB13 [23, 52], a variety of PLGA based nanoformulations that maintain the natural cannabinoid profile found in the plant, or full-spectrum formulations, are proposed and evaluated in the present work. For this main goal, three cannabis strains, containing different cannabinoid profiles, were used, and non-functionalized PLGA capsules together with pectin, alginate or chitosan coated capsules were prepared with each of them. In order to evaluate the overall features of the proposed formulations, to rule out possible inadequate formulations and to have a preliminary idea of their possible behavior in case of ingestion, a basic physicochemical characterization was carried. Thus, the cannabinoid content, particle-size, morphology and zeta-potential of the proposed formulations were evaluated, and the potential release profile and oral bioaccessibility of entrapped cannabinoids were studied by simulated *in vitro* digestion.

## Materials and Methods

### Materials and Standards

The PLGA (powder, 50:50) employed for the synthesis of nanocapsules was obtained from MedChemExpress (Monmouth Junction, NJ, USA). Polyvinyl alcohol (PVA, 13.000–23.000 g/mol, 98% hydrolyzed), alginic acid sodium salt from brown algae (low viscosity), chitosan (medium molecular weight), sucrose (for molecular biology,  $\geq 99.5\%$ ), pepsin from porcine gastric mucosa (powder,  $\geq 250$  units/mg solid, lot result: 444 U/mg), pancreatin from porcine pancreas (powder, suitable for cell culture, 4× USP specifications), bile salts (for microbiology) and sodium bicarbonate (ACS reagent,  $\geq 99.7\%$ ) were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany). Pectin was produced by Guinama (La Pobra de Vallbona, Spain) and phosphatidylcholine (Phospholipon® 90 G,  $\geq 94\%$ ) by Phospholipid GmbH (Cologne, Germany). Hydrochloric acid (HCl, 36%) was purchased from Merck KGaA (Dramstadt, Germany). Ethanol (99.5%) was obtained from Panreac Química S.L.U. (Barcelona, Spain) and ethyl acetate ( $\geq 99.8\%$ , ChromAR® for HPLC) and acetonitrile ( $\geq 99.8\%$ , ChromAR® for HPLC) from Macron Fine Chemicals (Gliwice, Poland). Activated charcoal (powder, reagent grade), methanol (UHPLC-MS grade) and water (UHPLC-MS grade) were purchased from Sharlab (Sentmenat, Spain), whereas Milli-Q quality water ( $< 0.05 \mu\text{S}\cdot\text{cm}^{-1}$ ) was produced using a Millipore 185 from Millipore (Burlington, MA, USA).

The solutions of individual standards of cannabinoids  $\Delta^9$ -tetrahydrocannabinolic acid-A (THCA, 1000  $\mu\text{g}/\text{mL}$  in methanol),  $\Delta^9$ -tetrahydrocannabinol (THC, 1000  $\mu\text{g}/\text{mL}$  in methanol)  $\Delta^9$ -tetrahydrocannabivarin (THCV, 1000  $\mu\text{g}/\text{mL}$  in acetonitrile),  $\Delta^9$ -tetrahydrocannabivarinic acid (THCVA, 1000  $\mu\text{g}/\text{mL}$  in acetonitrile), cannabichromene (CBC, 1000  $\mu\text{g}/\text{mL}$  in methanol), cannabichromenic acid (CBCA, 1000  $\mu\text{g}/\text{mL}$  in acetonitrile), cannabidiol (CBD, 1000  $\mu\text{g}/\text{mL}$  in methanol), cannabidiolic acid (CBDA, 1000  $\mu\text{g}/\text{mL}$  in acetonitrile), cannabigerol (CBG, 1000  $\mu\text{g}/\text{mL}$  in methanol), cannabigerolic acid (CBGA, 1000  $\mu\text{g}/\text{mL}$  in acetonitrile), cannabinol (CBN, 1000  $\mu\text{g}/\text{mL}$  in methanol) and cannabinolic acid (CBNA, 1000  $\mu\text{g}/\text{mL}$  in methanol), cannabidivarin (CBDV, 1000  $\mu\text{g}/\text{mL}$  in methanol), cannabidivarinic acid (CBDVA, 1000  $\mu\text{g}/\text{mL}$  in acetonitrile) were purchased by Dr. Ehrenstofer GmbH (Augsburg, Germany). The deuterated analogue  $\Delta^9$ -tetrahydrocannabinol (THC, 1000  $\mu\text{g}/\text{mL}$  in methanol) was supplied by Merck KGaA (Dramstadt, Germany) and phenantrene, used as internal standard (IS), was purchased from Sigma-Aldrich Chimie (Saint-Quentin-Fallavier). A mixed fresh stock solution containing 100  $\mu\text{g}/\text{mL}$  of

all target compounds was prepared monthly in methanol, whereas intermediate dilutions were prepared daily according to the experimentation.

### Preparation of Purified Cannabis Extracts

Cannabis flowers from three different cannabis strains were made into enriched cannabis extracts. The strains were chosen to be representative of the three most relevant cannabis chemotypes: (i) Chemotype I (CI) – a THC rich strain (~12% THC), (ii) Chemotype II (CII) – a THC/CBD leveled strain (~5% THC and ~7% CBD) and (iii) Chemotype III (CIII) – a CBD rich strain (~11% CBD). Flowers (around 15 g) of each strain were grinded and placed into separate closed Pyrex containers, and were maintained at 120 °C for 1 h to assure complete decarboxylation of acidic cannabinoids while keeping losses to a minimum [53]. After that, the Pyrex containers were stored at –40 °C, and once they were cooled down, cold ethanol (–40 °C) was added until the decarboxylated flowers were fully covered. Cold extraction of neutral cannabinoids was performed by maintaining the ethanol solutions in an ultrasonic bath for 1 min and, then 5 min at –40 °C. Once extraction was completed, ethanol extracts were vacuum filtered using 10–12 µm cellulose filters, and the resulting extracts were stored at –80 °C overnight to purify them by winterization [54]. The waxes, and lipids that precipitated during this period, were removed by a fast vacuum filtration using 0.45 µm cellulose nitrate filters. The remaining chlorophyll was removed by adding 80 mg of activated charcoal per initial cannabis flower gram (1.25 g for each extract approximately), vortexing for 10 min and filtering using 0.45 µm polypropylene syringe disk filters. Finally, the excess of ethanol was removed by a rotary evaporator (35 °C, 100 mbar) until viscous extracts were obtained (around 2 g per strain). The three extracts were stored at –20 °C until further use.

### Cannabinoid Content in Purified Cannabis Extracts

The concentration of main cannabinoids of each cannabis strain's extract was determined by means of high performance liquid chromatography coupled to a diode array detector (HPLC–DAD) in triplicate following a previously validated method [55]. Briefly, 0.1 g of cannabis extract were sonicated with 5 mL of methanol in an ultrasonic bath for 15 min. Subsequently, the solution was centrifuged for 5 min at 10000 g in a 5804 R Eppendorf centrifuge (Hamburg, Germany), and the supernatant solution was diluted with methanol containing IS (resulting in a concentration of 5 µg/mL IS in the final solution). The final solutions were syringe-filtered with 0.22 µm polypropylene disks and analyzed by means of HPLC–DAD using the method described in the following sections.

### Preparation of PLGA Nanoparticles

PLGA nanoparticles were prepared based on an emulsion solvent evaporation technique previously described for encapsulation of pure CBD [33] with some modifications due to the nature of the cannabis extracts. First, 12 PLGA solutions, each of them containing 500 mg of PLGA dissolved in 10 mL of ethyl acetate, were prepared. The PLGA-ethyl acetate solutions were loaded with cannabinoids by adding the different cannabis extracts. Owing to the lack of previous references related to cannabis extract containing PLGA formulations, four cannabis extract to PLGA powder mass ratios (referred as load ratio from now on) were tested for each of the cannabis strains (see Table I). Depending on this, 50, 150, 300 or 500 mg of each strain's extract were added to each of the 12 initial PLGA solutions (resulting in 4 loading ratios per strain). Once the extracts were dissolved, the PLGA-ethyl acetate-cannabis solutions were added dropwise into 50 mL of PVA 0.5% aqueous solutions, which

**Table I** Scheme and Codification of Synthesized PLGA Nanocapsules

Strain	Load ratio (mg <sub>extract</sub> :mg <sub>PLGA</sub> )	Coating			
		Uncoated	Alginate	Pectin	Chitosan
Chemotype I (CI)	1:10	CI10Ø	CI10A	CI10P	CI10C
	3:10	CI30Ø	CI30A	CI30P	CI30C
	6:10	CI60Ø	CI60A	CI60P	CI60C
	10:10	CI100Ø	CI100A	CI100P	CI100C
Chemotype I (CII)	1:10	CII10Ø	CII10A	CII10P	CII10C
	3:10	CII30Ø	CII30A	CII30P	CII30C
	6:10	CII60Ø	CII60A	CII60P	CII60C
	10:10	CII100Ø	CII100A	CII100P	CII100C
Chemotype I (CIII)	1:10	CIII10Ø	CIII10A	CIII10P	CIII10C
	3:10	CIII30Ø	CIII30A	CIII30P	CIII30C
	6:10	CIII60Ø	CIII60A	CIII60P	CIII60C
	10:10	CIII100Ø	CIII100A	CIII100P	CIII100C

were stirred at 24000 rpm by an Ultra-turrax (IKA, Staufen, Germany) in order to emulsify nanoparticles. This process was carried out for all formulations except for the highest loading ratio (i.e., 10:10) since an indivisible agglomerate that blocked the Ultra-turrax was formed during the process. After emulsification, excess of ethyl acetate was removed by rotary evaporation (40 °C, 100 mbar) while excess of PVA and non-encapsulated cannabinoids were removed by centrifugation and decantation for three times by adding further Milli-Q water. The obtained wet PLGA-cannabis nanoparticles for each strain and load were separated in four aliquots for further functionalization. One of the aliquots per load and strain was stored at 4 °C (uncoated capsules) and the other three were functionalized using different coating layers: alginate, pectin and chitosan (see Table I). The functionalization of those PLGA-cannabis nanoparticles was carried out by the addition of 10 mL of 0.2% aqueous solutions of the corresponding coating agent (i.e., sodium alginate, pectin, and chitosan) onto each of the aliquots and continuously agitated at room temperature for 2 h. Then, Milli-Q water was added and the excess of coating polymer, along with potential traces of non-encapsulated cannabinoids were removed through centrifugation and decantation. This process was repeated three times by adding further Milli-Q water. Once cleaned, 10 mL of cryoprotecting solution (i.e., sucrose 1% aqueous solution [56]), was added to each of the 4 fractions (non-functionalized and functionalized) for each strain and load; and were dried subsequently in a Coolvacuum Lyomicron freeze-dryer (Barcelona, Spain) at -60 °C and 0.037 mbar for 48 h. The sucrose was separated and the obtained dry capsules were weighed and stored at -20 °C until further use.

### Cannabinoid Content in PLGA Nanoparticles

The concentration of 14 cannabinoids in the synthesized PLGA-cannabinoid nanoparticles was determined in triplicate by means of HPLC–DAD following a method described in the literature used to determine the content of a synthetic cannabinoid in PLGA nanoparticles [37]. 1 mL of acetonitrile was added onto accurately weighed 4 mg of lyophilized PLGA nanoparticles, and the mixture was vortexed (5 min) to dissolve PLGA nanoparticles and promote liberation of entrapped compounds in the organic phase [37, 57–59]. The obtained acetonitrile solutions were diluted in methanol containing IS (resulting in a concentration of 5 µg/mL in the final solutions) and filtered using 0.22 µm polypropylene disks. The cannabinoid content in the final solutions was determined by means of HPLC–DAD analysis.

5 µL of the final solutions were analyzed using an Infinity 1260 LC System (HPLC) coupled to an Infinity 1260 Diode Array Detector WR (DAD), both from Agilent Technologies (Santa Clara, CA, USA). Separation of cannabinoids was

achieved using a Kinetex C18 column (150×3 mm, 2.6 µm) with a Security Guard Ultra C18 precolumn (2×3 mm), both from Phenomenex (Torrance, CA, USA). The separation of cannabinoids was done using a gradient method at a constant flow of 0.7 mL/min consisted of mobile phases A (Water, 0.1% acetic acid) and B (Methanol, 0.1% acetic acid). The gradient method started at 30% A and maintained 3 min; then, it was decreased first to 20% in 6 min and then to 5% in 3 min, which was maintained for 3 min. A was increased to 30% in 5 min and maintained for other 4 min to reach initial conditions before the next chromatographic run, which last 24 min in total. Cannabinoids were detected using DAD and were quantified at 230 nm using an external calibration curve prepared in the range of 0.1 µg/mL and 50 µg/mL for all target compounds. All the calibration solutions, as well as the measured extracts, contained IS in a concentration of 5 µg/mL in order to correct the DAD signal and minimize instrumental measurements variability.

### Encapsulation-efficiency

The encapsulation-efficiencies (EE %) for each formulation and cannabinoid were determined by comparing encapsulated mass ( $mg_{cap}$ ) of each cannabinoid with the mass of cannabinoid that was loaded along with the cannabis extract ( $mg_{ext}$ ) (Eq. 1):

$$EE \% = \frac{mg_{cap}}{mg_{ext}} = \frac{m_{cap} \cdot C_{cap}}{m_{ext} \cdot C_{ext}} \quad (1)$$

where,  $m_{cap}$  is the obtained mass in g of nanoparticles,  $C_{cap}$  is the concentration in mg/g of each cannabinoid in each formulation,  $m_{ext}$  is the employed mass in mg of cannabis extract and  $C_{ext}$  is the concentration of each cannabinoid in mg/g in each of the cannabis extracts.

### Physical Characterization of PLGA Nanoparticles: Particle Size, Zeta-Potential and SEM Imaging

The particle size and zeta-potential of PLGA nanocapsules were measured using a Zetasizer Nano series from Malvern Instruments (Malvern, UK). For this purpose, 4 mg of dried PLGA nanocapsules were suspended in 4 mL of Milli-Q water under continuous magnetic stirring to promote homogeneity of the suspension. Aliquots of the homogenous dispersions were used to prepare dilutions of 0.05 mg/mL (particle size) and 0.5 mg/mL (zeta-potential). The particle size and zeta-potential of three aliquots of the final dilutions were determined respectively by dynamic light scattering and electrophoretic light scattering (at an angle of 90°).

The morphology of the synthesized nanocapsules was checked by means of Scanning Electron Microscopy (SEM). The dried PLGA nanocapsules were coated with 15 nm of

gold on a K550X sputter coater from Emitech (Montigny-le-Bretonneux, France) and measured using a FEG SEM S4800 from Hitachi (Tokyo, Japan) with an acceleration voltage of 5 kV.

### In Vitro Gastro-Intestinal Digestion Simulation

Release of the main cannabinoids was determined by a static *in vitro* digestion simulation, following the recommendations of INFOGEST [60] and Minekus et al. [61]. Simulated gastric fluid (SGF) contained 2000 U/mL of pepsin and 0.17 mM of phosphatidylcholine in Milli-Q water and the pH was adjusted to 3 using a 2 M HCl solution. Simulated intestinal fluid (SIF) contained 3.2 g/L of pancreatine and 8.16 g/L of bile salts in Milli-Q water. The content of main cannabinoids (THC and CBD) in dry PLGA nanocapsules was considered to standardize the assays and provide an easier comparison between formulations. The mass employed in each assay was the one required to provide 10 mg of THC (CI nanoparticles), 10 mg of CBD (CIII nanocapsules) or 10 mg of THC + CBD (CII capsules).

For each formulation the calculated PLGA nanoparticles mass was weighed and 10 mL of SGF were added and maintained at 37 °C under continuous stirring for 2 h, simulating the gastric phase. After that, 10 mL of SIF were added (resulting in 1.6 g/L of pancreatine and 4.08 g/L of bile salts), pH was adjusted to 7 using a saturated sodium bicarbonate aqueous solution, and the mixture was maintained at 37 °C under continuous stirring for 4 h, simulating the intestinal phase. The release profile of cannabinoids was determined by taking aliquots of 200 µL at different timings (12 aliquots during the 6 h of the experiment). Each time the aliquots were taken, aliquots of 200 µL of fresh SGF or SGF:SIF 1:1 mixture were added to the assay-solution in order to maintain the same ratio within microcapsules and simulated fluids in the whole simulation. All the assays were run in triplicate. All the aliquots were diluted with methanol containing IS (resulting in a concentration of 5 µg/mL IS in

the final solution) and centrifuged using a 5424 R Eppendorf Centrifuge (Hamburg, Germany) for 5 min at 10000 g. The supernatants were collected and filtered with 0.22 µm polypropylene syringe disks prior to HPLC–DAD analysis.

The results were expressed as the released cumulative fraction from the total cannabinoid content through digestion time. Intestinal bioavailability was calculated by resting the fraction released in the gastric phase to the final released fraction.

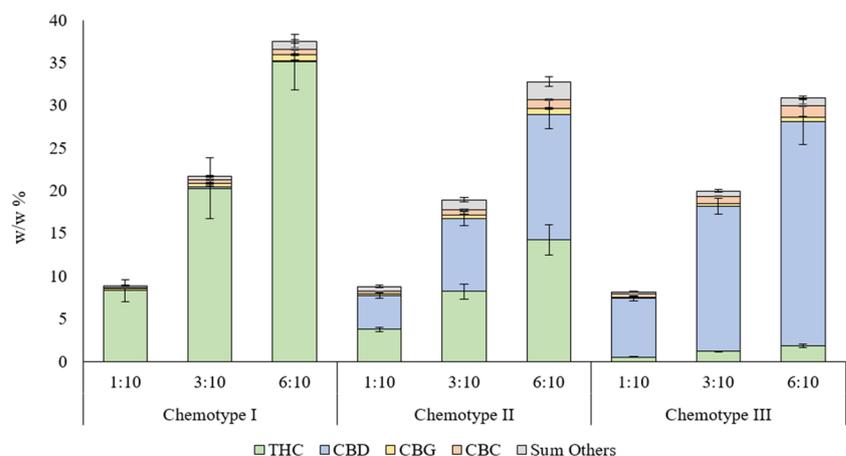
## Results

### Cannabinoid Content and Encapsulation-efficiency

The content of 14 cannabinoids in the synthesized PLGA nanoformulations is compiled in Table S1. Since the applied polymeric coating did not result in significant differences ( $p$ -value > 0.05) in the cannabinoid content of capsules made with the same cannabis strain and extract loading, the mean content of the uncoated and three coated formulations per strain and extract loading are shown in Fig. 1. The cannabinoid content in the obtained PLGA nanocapsules are in line with the cannabinoid profile of the employed extract and the loaded extract amount in each case (cannabinoid content of the extracts are shown in Table S2).

CI capsules, contained high amounts of THC, between 8 and 35% of the capsules' mass, and contained CBD, CBC, CBG, CBN and THCV (together with their acidic analogs) in concentrations between 0.01% and 0.7%. This made CI capsules to have the highest content of cannabinoids and to have the widest spectrum of neutral cannabinoids, compared to the capsules obtained from CII and CIII strains. CII capsules, contained between 3.8% and 14% of THC and between 4.0% and 15% of CBD, and the content of CBC, CBG and CBN (together with their acidic analogs) were encountered in concentrations between 0.01% and 1.16%. CIII capsules, on the other hand, contained high amounts of CBD, between

**Fig. 1** Content of THC, CBD,CBG,CBC and sum of other minor cannabinoids (CBN, THCV, THCA, CBDA, CBGA and CBCA) in PLGA nanocapsules (w/w %) for each cannabis strain and load ratio ( $\text{mg}_{\text{extract}}:\text{mg}_{\text{PLGA}}$ ) ( $n = 12$ , expressed at a 95% confidence level)



6.9% and 26% of the capsules' mass and contained CBC and CBG in concentrations between 0.01% and 1.3%. Thus, the total cannabinoid content in CII and CIII is slightly lower, and although the cannabinoid spectrum is tighter, the content in minor cannabinoids such as CBC and CBG, is higher than in CI capsules. Considering that the average oral dose is about 30 mg for THC, 60 mg for THC + CBD (30 mg of each) and about 100 mg for CBD [7, 62, 63], between 360 and 80 mg of CI capsules, 790 and 200 mg of CII capsules and 1400 and 400 mg of CIII capsules would be enough to ensure those doses.

In addition to the expected differences related to the nature of the cannabis strain and the extract load, some similarities can be highlighted. The content of acidic cannabinoids in all the formulations is minimum, especially the content of THCA (even in high THC content capsules) due to its lower decarboxylation temperature [53]. This observation indicates that the decarboxylation process carried in the preparation of the three cannabis extracts was appropriate. Similarly, low content of CBN (the main degradation product of THC's oxidation) was observed in all capsules, which is a fact of great interest in the case of CI and CII capsules (both containing high amounts of THC). Based on this result, we can conclude that the employed cannabis flowers were fresh (the higher CBN content is, the older a cannabis product is) and that THC was not oxidized neither in the preparation of the cannabis extracts nor in the encapsulation process [64, 65]. This could be achieved by avoiding high temperatures (after decarboxylation), prolonged exposure to light and exposure to oxidant species.

THCVA, CBDV and CBDVA were under the method limits of detection for the measured dilutions (0.01%). Although the concentration of these minor cannabinoids could have been determined by measuring a higher concentration extract, this option was discarded since the required dilution to measure these cannabinoids, based on the low content in the purified extracts (see Table S2), would saturate the column and the HPLC system.

Concerning the encapsulation efficiencies (EE) of major cannabinoids, a slight decrease was observed as the cannabis extract loading was increased in all formulations (Table II). The highest EEs were obtained in nanocapsules loaded with 10 mg of cannabis extract per 100 mg of PLGA, where mean EEs between 85 and 99% were obtained. When the cannabis extract load was increased to 3:10, the mean EE decreased to values between 65 and 84%, and when increased to 6:10, the

EE % decreased to values between 56 and 73%. Likewise, Martin-Banderas et al. and Fraguas-Sanchez et.al reported lower EE % of CB13 or CBD in their PLGA based encapsulations when cannabinoid load was increased [33, 37], which is attached to capsules' maximum loading capacity or to loss of available drug due to its migration into the emulsifier aqueous phase. In contrast to this phenomenon, no significant differences were observed in the EE % of different strains and cannabinoids.

### Physical Properties of PLGA Nanocapsules: Particle Size, Zeta-potential and SEM Imaging

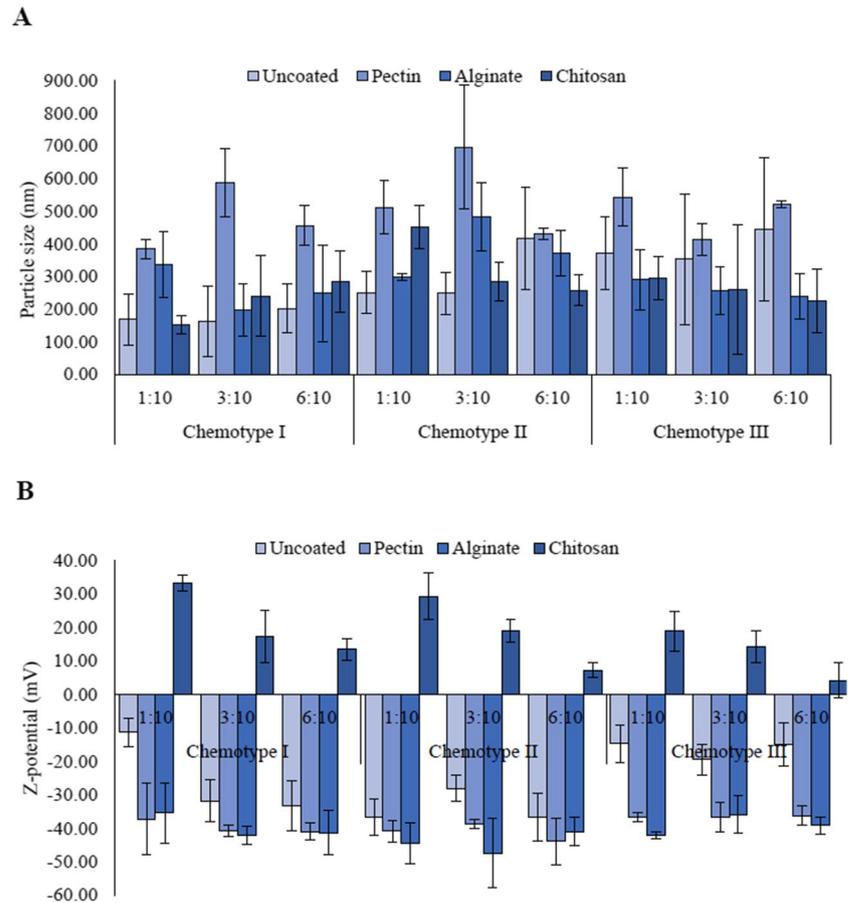
Particle size and zeta-potential of PLGA nanoparticles were determined using a zeta-sizer, measurement plots of both parameters are shown in Figures S1 and S2 respectively. The polydispersity index (PDI) values of most formulations were around 0.3 (Table S3), indicating that they had an acceptable dispersity [66]. For this reason, and according to ISO 22412:2017, Z-average was elected to report the mean particle size of each formulation [67]. The particle size ranged between 150 and 700 nm, being the applied coating in each formation the possible responsible of such variability (Fig. 2A). In fact, particles with the same coating showed statistically comparable particle sizes, being the pectin coated capsules the largest ones (380–700 nm) compared to either uncoated (160–440 nm), alginate coated (200–480 nm) or chitosan coated (150–450 nm) capsules (depending on the case). On the contrary, no clear differences were observed among strains and extract load ratios. Uncoated, alginate coated and chitosan coated nanocapsules showed comparable particle sizes among them in most cases. The obtained particle sizes fall into the same size range than those obtained in THC, CBD or CB13 loaded PLGA nanoparticles in previous works, which ranged between 250 and 450 nm in non-functionalized particles and between 600 and 900 nm in functionalized particles [52].

The success in the surface-modification (or coating) process and its effect on the surface-charge of nanoparticles was assessed using the zeta-potential values (Fig. 1b). As expected, uncoated, pectin coated and alginate coated capsules showed negative zeta-potentials due to their anionic nature, whereas chitosan coated capsules showed positive zeta-potentials due to their cationic nature. The addition of pectin and alginate, increased significantly the negative charge of uncoated PLGA capsules in CI 1:10 and 3:10

**Table II** Encapsulation-efficiencies (EE) of THC and CBD for Each Strain and Load Ratio (n = 12, 2 s at 95% Confidence Level)

Strain	CI			CII			CIII		
	1:10	3:10	6:10	1:10	3:10	6:10	1:10	3:10	6:10
THC (EE %)	98 ± 9	84 ± 13	73 ± 4	99 ± 5	76 ± 7	66 ± 8			
CBD (EE %)				87 ± 5	65 ± 6	56 ± 6	85 ± 3	73 ± 1	57 ± 2

**Fig. 2** Mean particle size (A) and zeta-potential (B) of PLGA nanoparticles for each strain, extract load ratio ( $\text{mg}_{\text{extract}}:\text{mg}_{\text{PLGA}}$ ) and coating ( $n=3$ , expressed at 95% confidence level)



loaded capsules, CII 3:10 loaded capsules and all CIII capsules. Interestingly, in all cases (except for CIII 1:10 loaded capsules) the zeta-potential of pectin and alginate were comparable between them ( $p > 0.05$ ), as well as comparable among nanocapsules derived from different extract load ratios and cannabis strains. In the case of uncoated capsules, despite no clear tendency was observed, significant differences were observed among formulations, since CI 1:10 loaded, and CIII capsules showed less negative zeta-potentials compared to the rest. For this reason, surface modification using pectin and alginate, seems a good strategy to increase negative zeta-potential and to standardize the surface-charge of formulations, regardless of the strain and extract loading ratio. Indeed, pectin and alginate coated capsules showed zeta-potential values more negative than  $-30$  mV in all formulations, which technically ensures colloidal suspension stability and prevention of particle aggregation and flocculation [68]. Regarding the possible interaction of capsules with the intestinal mucosa, anionic polymers such as alginate and chitosan have proven to enhance the retention of capsules in the intestine by adhesion mechanisms to the intestinal mucosa, leading to increased intestinal absorption of encapsulated compounds [69–71]. Although the intestinal membrane is negatively charged, the

carboxylic groups of pectin and alginate can form hydrogen bonds with oligosaccharide chains of intestinal mucins [72]. These interactions along with chain entanglement and van der Waal's interactions, are stronger than electrostatic repulsion, and result in high mucoadhesion of these biopolymers [73, 74]. Interestingly, a higher negative charge density in these polymers is related to the mucoadhesive strength and hence, the obtention of such negative zeta-potentials suggests that mucoadhesion and absorption of cannabinoids may be higher in pectin- and alginate-coated formulations compared to uncoated capsules [75, 76].

On the other hand, more differences were observed in the positive zeta-potentials of the capsules coated with chitosan. CI and CII 1:10 loaded capsules showed significantly higher zeta-potentials compared to CIII 1:10 loaded capsules, and CI 6:10 loaded capsules showed significantly higher values compared to CII and CIII 1:10 loaded capsules; whereas 3:10 loaded capsules showed comparable zeta-potentials regardless of the strain. Thus, CIII capsules showed slightly lower positive zeta-potentials compared to CI and CII capsules. However, the cannabis extract loading employed was the most critical factor that appeared to negatively influence the zeta-potential value of chitosan-coated capsules. Increasing the loading ratio of cannabis extract led to significant

decreases in the zeta-potential values of the chitosan-loaded capsules in all strains. Overall, zeta-potential values above + 30 mV were only achieved with 1:10 loaded CI and CII capsules, suggesting that the remaining chitosan-coated formulations would not result in stable aqueous dispersions, and mucoadhesive or mucopermeable properties might also be compromised [68]. The improved intestinal adhesion and permeability of chitosan (and other cationic polymers) coated capsules is the result of the electrostatic attraction between the positive charge of chitosan and the negative charge of intestinal membranes [77, 78]. A less positive surface charge of the capsules could compromise electrostatic attraction between capsules and intestinal membranes, and therefore, formulations with higher cannabis extract load (and less positive zeta-potentials) may exhibit less efficient absorption and a lower bioavailability of cannabinoids [75, 79].

In addition to particle-size and zeta-potential, the morphology of PLGA nanocapsules was evaluated by SEM analysis. Considering the minor influence of the nature of the employed cannabis strain in particle-size and zeta-potential, it was assumed that morphology of capsules would not vary significantly between capsules derived from different cannabis chemotypes. Hence, SEM images of all the formulations of one of the chemotypes (chemotype II) were taken and assumed to be representative for equivalent formulations (i.e. same extract load ratio and coating) of the other chemotypes (Figure S3). SEM images revealed certain heterogeneity in the morphology of nanocapsules in most formulations. Although spherical or quasi-spherical shapes were predominant, elongated and irregular shapes were also observed. The polymeric coating used did not produce conclusive differences in the shape of capsules. The extract loading ratio did not lead to clear differences in morphology neither, but appeared to be related to the agglomeration of nanocapsules. Regardless of the polymeric coating, a higher degree of agglomeration is observed with the increase of the loading ratio. This observation is in line with the fact that 10:10 loaded capsules could not be processed due to the formation of big agglomerates. This phenomenon led to the formation of some nanoparticle-networks in 6:10 loaded capsules, as it was also observed in the literature [51]. However, these agglomerations in dry nanocapsules seem to be reduced or to disappear when capsules are dispersed in water owing to the observed particle sizes and zeta-potential (and the measurement plots of both parameters). In regard to the surface, capsules with 1:10 extract load ratio seemed to have a smoother surface compared to capsules with higher loading ratios, but overall capsules presented rough and irregular surfaces.

Considering the observed tendencies and comparing the overall capsule morphologies with the spherical, regular and smooth surfaces obtained by Fraguas-Sanchez et. al, whose

particle synthesis protocol was followed, it seems that the use of cannabis extracts instead of pure CBD, may influence negatively the morphology of capsules [33]. The observed angular and irregular morphology in some capsules could negatively affect their transport and membrane permeation features, and the lack of homogeneity may compromise the homogenous release of encapsulated compounds [80–82].

Although morphology of some nanocapsules could be improved, most formulations showed satisfactory particle sizes (confirmed by SEM imaging) and zeta-potentials, and so, release and absorption of PLGA nanocapsules may still be suitable for a proper delivery of encapsulated cannabinoids [83, 84]. However, the use of lower cannabis extract loading ratios is recommended to assure the integrity of PLGA nanocapsules structure and the effectivity of the functionalization if chitosan is used. Similarly, if functionalization via anionic polymers is intended, the use of alginate is recommended over pectin, as pectin coating seemed to lead to a higher particle size in some cases.

### ***In Vitro* Gastro-intestinal Digestion Simulation: Release Profile and Bioaccessibility**

*In vitro* gastro-intestinal digestion simulations were carried in order to get a preliminary idea of the release-profile and intestinal bioaccessibility of cannabinoids in the proposed PLGA nanoformulations. Moreover, these assays allow determining whether the employed cannabis strain, extract load ratio and coating has any effect on the release of cannabinoids of PLGA nanocapsules in gastro-intestinal media or not.

The release-profile of each formulation was determined based on the cumulative release of main cannabinoids (THC, THCA and CBD, and CBD, in CI, CII and CIII strains respectively) during the *in vitro* gastro-intestinal digestion simulation. The raise of cumulative release during simulated digestion was graphically represented for each formulation (Figure S4). All formulations followed a sustained release profile of cannabinoids in both gastric and intestinal phases. In some cases though, the sustained release in the intestinal phase was preceded by a narrow burst release at the beginning of the intestinal phase. Despite the similarities in the release profiles, there are differences in release rate of main cannabinoids depending on the formulation. With the aim of having a deeper insight into the *in vitro* release profiles of the formulations studied in this work release kinetic models were built and compared. According to the literature, release mechanisms of drugs from PLGA nanocapsules are based on zero-order, first-order, Higuchi's and Korsmeyer-Peppas' Eqs. [85–88]. In this work, zero-order, first-order and Higuchi's kinetic models were tested, which are common and basic kinetic models that work for most formulations,

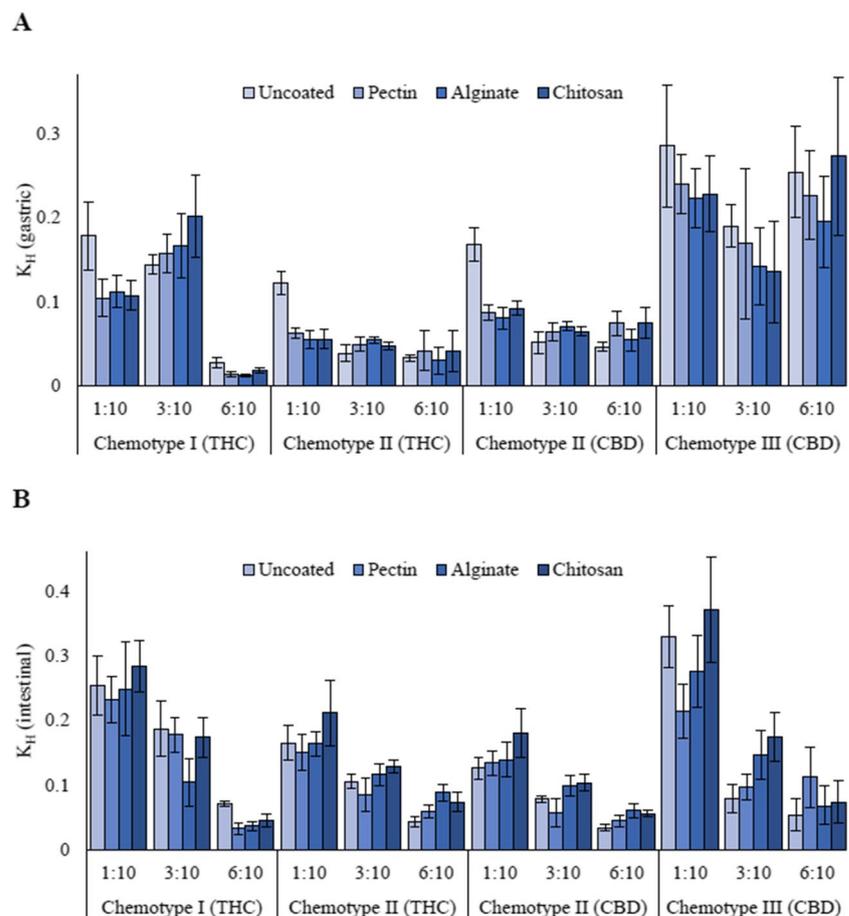
and give one kinetic constant per model, which eases the comparison among formulations [89]. The release data was split to obtain independent kinetics for the gastric and intestinal phases.

Higuchi's kinetic model described best the cumulative release of cannabinoids from PLGA nanocapsules in most cases (81 out of 96). Although first-order kinetic model fitted better to the release of cannabinoids in the gastric phases of some formulations, Higuchi's model had a similar fitting in most cases (see Table S4). This model fitted adequately to the release in both gastric and intestinal phases and the obtained coefficients of determination ( $r^2$ ) were above 0.9 in 83 out of 96 models (and above 0.95 in 65 out of 96 models). Those coefficients were only below 0.9 in most CIII 6:10 loaded formulations ( $r^2 > 0.8$ ) and in the gastric phases of CII60P, CII60A and CII60C capsules for THC ( $0.73 < r^2 < 0.78$ ). These results suggest that, in most of the synthesized nanocapsules, cannabinoids follow a fickian diffusion that fit to Higuchi's kinetic model [89]. For those poor fitting curves, cannabinoids may follow a quasi-fickian or non-fickian diffusion that could better fit to Higuchi-derived models, such as Korsmeyer–Peppas' model, as it has been previously reported [26, 86, 90]. In any case, the obtained release constants from Higuchi's model ( $K_H$ ) in each phase

(gastric and intestinal) were used to compare the release velocities among formulations.

The  $K_H$  values of the gastric phase (Fig. 3A), show that the polymeric coating and the extract load ratio led to little or no significant differences among formulations. The  $K_H$  values of formulations made with the same cannabis strain and extract load but different coating, did not differ statistically ( $p > 0.05$ ), except for (i) 1:10 and 6:10 loaded CI capsules and 1:10 loaded CII capsules, where uncoated capsules showed higher  $K_H$  values compared to coated ones, and (ii) 6:10 loaded CII capsules, where uncoated capsules showed lower  $K_H$  values compared to pectin and chitosan coated ones. The extract load ratio led to significant differences in the release speed of CI capsules (1:10 and 3:10 loaded had higher  $K_H$  compared to 6:10 loaded) and in CII uncoated and chitosan coated capsules (1:10 loaded had higher  $K_H$  compared to 3:10 and 6:10 loaded ones), but did not lead to any significant difference in the rest of formulations. In contrast, the employed cannabis strain did lead to significant differences in most cases. Regardless of the extract load and polymeric coat, CIII capsules showed significantly higher  $K_H$  values compared to CI and CII capsules, except for CII00, CII30P, CII30A and CII30C capsules, which had comparable release speeds compared to their CIII analogous formulation.

**Fig. 3**  $K_H$  values for the release of main cannabinoids from PLGA nanocapsules in **A** gastric and **B** intestinal phases; for each strain, extract load ratio ( $m_{\text{extract}}:m_{\text{PLGA}}$ ) and coating ( $n=3$ , expressed at 95% confidence level)



Additionally, CI capsules showed faster releases compared to CII capsules, excepting 6:10 loaded capsules where the contrary trend was observed.

Intestinal  $K_H$  values (Fig. 3B), were statistically higher compared to the gastric  $K_H$  values, probably due to the burst release observed in the beginning of the intestinal phase. There were a few exceptions though, such as CI 3:10 loaded, CII 6:10 loaded (only for THC) and CIII 3:10 loaded capsules where not show significant differences among digestive phases were observed, and CII (only for CBD) 6:10 and CIII 6:10 loaded capsules, which showed lower intestinal  $K_H$  values.

Compared to the gastric phase, the used cannabis strain did not lead to significant differences in the release speed of cannabinoids in the intestinal phase. Although CIII0, CIII0A, CIII0C, CII300 and CIII0C capsules had a significantly slower release compared to analogous CI and CIII formulations, the  $K_H$  values of capsules with the same load and polymeric coat were comparable in the rest of cases for all the chemotypes. Similar to the gastric phase, the applied polymeric coat did not lead to significant differences in the intestinal release speed generally. In contrast, the extract load ratio, did lead to significant variations in all formulations. Regardless of the employed cannabis strain and polymeric coat, 1:10 loaded capsules showed significantly higher  $K_H$  values compared to 3:10 loaded capsules in all cases (except for CI uncoated and pectin coated capsules); and 3:10 loaded capsules showed significantly higher  $K_H$  values compared to 60% loaded capsules (except CII for pectin capsules and CIII uncoated and pectin capsules). This suggests that the release speed decreases as the extract load ratio increases.

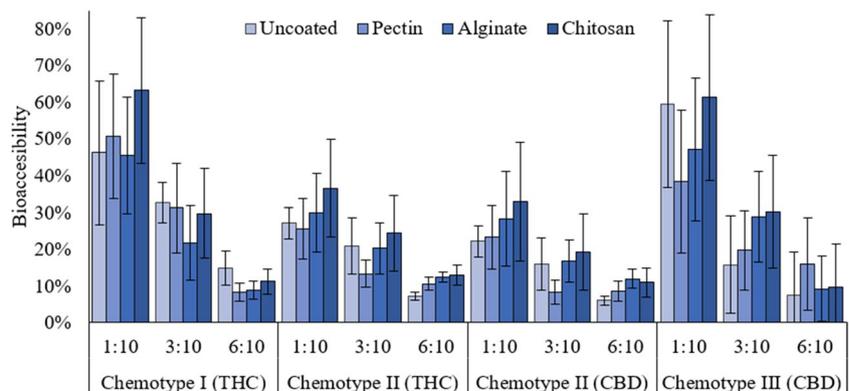
The potential intestinal bioaccessibility of each formulation was estimated by subtracting the fraction released at the end of the gastric phase to the cumulative release at the end of the *in vitro* gastro-intestinal simulation. The obtained *in vitro* bioaccessibility values ranged from 6 to 63%. The observed differences due to the variables (i.e., cannabis strain, extract load ratio and polymeric coat) of the formulations match with the tendencies observed in the  $K_H$  values

in the intestinal phase (Fig. 4). Analogously, nor the applied polymeric coat (except 6:10 loaded CII derived capsules, where uncoated capsules showed lower bioaccessibility values), neither the applied cannabis strain (except some cases where CII capsules showed lower bioaccessibility values) led to any significant changes in the bioaccessibility of THC and CBD. In contrast, lower bioaccessibility values were obtained for capsules loaded with higher cannabis extract to PLGA mass ratios. In fact, bioaccessibility values of 1:10 loaded capsules were in all cases (except in pectin coated CIII derived capsules) statistically higher ( $p$ -value < 0.05) than the values obtained for 6:10 loaded capsules.

The observations found for  $K_H$  and bioaccessibility indicate that the cannabis extract loading has a significant effect on the release of cannabinoids from PLGA nanocapsules in gastro-intestinal media, rather than the employed cannabis strain or the functionalization via different polymers. In this context, it is worth mentioning the macroscopic differences observed when dry nanocapsules were handled for the *in vitro* bioaccessibility assays, which correspond to the structural differences observed in SEM images (Figure S3).

The appearance of capsules derived from different chemotypes or polymeric coatings were indistinguishable, whereas the extract load did led to noticeable changes. PLGA nanoparticles with 1:10 cannabis extract load were light brown color fine powders, but, as the cannabis extract load increased, nanocapsules capsules were darker and, more remarkably, got denser and stickier. In other words, as the cannabis extract load was increased the appearance of capsules got more similar to the employed cannabis extracts themselves, which were dark viscous and sticky oils as is the custom [91]. Similarly, dry PLGA nanocapsules loaded with higher amounts of cannabis extracts, seemed to be stuck in agglomerates in the SEM images (Figure S3). These observations suggest that increasing cannabis extract load ratio may decrease porosity while increasing density. These structural changes can hinder the entrance of water and thus, the hydrolysis of PLGA, as well as blocking the diffusion of cannabinoids through the pores [92]. All these processes

**Fig. 4** *In vitro* bioaccessibility of major cannabinoids in PLGA nanocapsules for each strain, extract load ratio ( $\text{mg}_{\text{extract}}:\text{mg}_{\text{PLGA}}$ ) and coating ( $n = 3$ , 95% confidence level)



can decrease the release rate of entrapped compounds, which may explain the differences observed for  $K_H$  and bioaccessibility values for capsules with different loading values. Similarly, the higher porosity in less loaded capsules, may explain the faster release of 1:10 loaded uncoated capsules compared to coated ones in the gastric phase, where PLGA hydrolysis is accelerated due to acidic catalysis [93]. Thus, in 1:10 loaded formulations the presence of an additional polymeric layer seems to decelerate the acidic catalyzed hydrolysis of PLGA, whereas in 3:10 and 6:10 loaded capsules the cannabis extract load to PLGA ratio seems to establish the release rate of cannabinoids.

Based on the results of the bioaccessibility assays, it can be concluded that loading PLGA nanocapsules with lower cannabis extract amounts may be more suitable in terms of effective intestinal dose, at least in *in vitro* conditions. The sticky and dense nature of the cannabis extract may hinder the release of cannabinoids through digestion. Due to this, cannabinoids remain entrapped in PLGA nanocapsules with higher extract loads, which results in a lower released dose in intestinal media, despite having a higher cannabinoid content.

The observed behavior of PLGA nanocapsules in simulated gastro-intestinal media and their physical characteristics suggest that capsules loaded with a 1:10 extract/PLGA ratio might be suitable for therapeutic applications. These capsules showed acceptable morphology, sufficient surface charge to provide colloidal stability and improved mucoadhesivity or mucopermeability, and suitable particle size in all cases, regardless of the polymeric coating applied or the nature of the cannabis strain. These formulations showed high intestinal bioaccessibility and sustained release of cannabinoids, and their particle-size and zeta-potential were similar to the above-mentioned CB13 PLGA nanocapsules designed for oral administration, suggesting that their performance after oral ingestion could be similar [34–37].

Even though these similarities suggest that cannabinoid release, transport and absorption could be improved in comparison to other cannabinoid containing edibles, further characterization should be carried to confirm such hypotheses and elucidate real pharmacokinetics and bioavailability of the proposed formulations. *In vivo* experiments would be the most suitable approach to obtain such information [94]. However, cellular uptake of cannabinoids could be assessed using human colon adenocarcinoma (Caco-2) cell models, which is broadly accepted as a way to mimic intestinal absorption [95, 96]. This could be particularly interesting in order to assess the mucopermeability and mucoadhesivity properties of the obtained PLGA nanocapsules, which could be substantially different among uncoated, alginate, pectin or chitosan coated nanocapsules, and could confirm the suitability of the size, shape and surface charge of nanocapsules [35, 97].

## Conclusions

The value of full-spectrum formulations has gained visibility in recent years compared to pure THC, pure CBD or THC and CBD formulations. This fact, together with the suitability of PLGA-based systems for trapping cannabinoids and providing selective, sustained and effective cannabinoid release, has been the starting point for developing a series of PLGA-full-spectrum cannabis nanoformulations designed for oral intake.

For this purpose, full-spectrum cannabis extracts were obtained from natural sources, and have been encapsulated in PLGA nanovehicles following procedures to encapsulate pure cannabinoids. In order to cover a wide range of potential therapeutic uses and different formulation possibilities, we studied the use of three different cannabis strains and the use of three common polymeric coatings (alginate, pectin and chitosan) along with uncoated capsules. Owing to the lack of previous references using cannabis extracts for nanoencapsulation using PLGA, four cannabis extract mass to PLGA mass ratios were tested. All these variables would result in 48 different formulations. However, as the preparation of 12 of them was not feasible (the ones with 10:10 cannabis extract to PLGA load), 36 different nanocapsules were finally synthesized. Cannabinoid content, basic physical characterization and release of cannabinoids in simulated gastro-intestinal media were determined in order to get a preliminary idea of their suitability.

The cannabinoid content of THC and CBD was high in all formulations, and owing to the use of three different cannabis chemotypes, the proposed formulations could cover a wide range of therapeutic applications. Moreover, compared to other cannabinoid containing preparations, the proposed formulations maintained small amounts of CBC, CBG and other minor cannabinoids, which could result in the entourage effect often sought in cannabis based products. Related to this, the nature of the cannabis strain did not lead to major differences in most of the studied properties, and so, it seems that the proposed formulations may be extended to additional strains if different cannabinoid profiles were sought.

Whereas the nature of the cannabis strain did not lead to significant differences, it was found that the amount used for the preparation of particles was critical. Indeed, when larger cannabis extract loads were used, the encapsulation efficiency, the functionalization with chitosan, the morphology and the release of cannabinoids in gastro-intestinal media was compromised. The viscous and dense nature of the cannabis extracts seemed to influence negatively the structure of capsules and obstruct significantly the release of cannabinoids, resulting in a substandard intestinal

accessibility of free cannabinoids. Therefore, lower cannabis extract load based formulations are recommended for a better performance of PLGA nanocapsules.

The applied polymeric coatings on the other hand, did not lead to appreciable differences in most of the studied properties, but seemed suitable to improve mucoadhesiveness and mucopermeability compared to uncoated PLGA nanocapsules. Moreover, the assessed properties of capsules were generally comparable to those obtained in single cannabinoid loaded PLGA nanoformulations found in the literature, suggesting that the proposed formulations could have a similar *in vivo* performance.

Nonetheless, despite the potential benefits that the proposed formulations could have for oral cannabinoid administration, further assays related to their pharmacokinetics and bioavailability are needed in order to evaluate their suitability for medicinal purposes. In any case, the exposed results are useful as a starting point to develop new PLGA nanocapsules loaded with full-spectrum cannabis extracts.

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## Declarations

**Competing Interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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