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UMA ATUALIZAÇÃO DE SISTEMAS NANOPARTICULADOS PARA A LIBERAÇÃO CONTROLADA DE CANABINOIDES: ASPECTOS BIOFARMACÊUTICOS & APLICAÇÕES TERAPÊUTICAS

AN UPDATE OF NANO-BASED DRUG DELIVERY SYSTEMS OF CANNABINOIDS: BIOPHARMACEUTICAL ASPECTS & THERAPEUTIC APPLICATIONS

Florianópolis, julho de 2022.

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Trabalho de Conclusão de Curso apresentado como um dos requisitos para a obtenção do grau de Bacharel em Farmácia.

Orientador: Prof^{o}. Dr. Thiago Caon

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RESUMO EXPANDIDO

A *Cannabis sativa* possui mais de 100 compostos fitocanabinóides em sua composição, tendo o THC e o CBD como principais representantes. Com a descoberta do sistema endocanabinóide no final do século XX, intensificaram-se pesquisas com estes ativos. Além de receptores canabinoides acoplados à proteína G, ligantes endocanabinoides e enzimas reguladoras compõe este sistema. Os receptores são expressos em diversos locais do corpo humano tais como sistema nervoso central, periférico, endócrino e imunológico. São responsáveis por diversas funções fisiológicas e contribuem para explicar a ampla gama de ações farmacológicas dos compostos que agem seletivamente nesta via. O THC atua como agonista parcial dos receptores canabinoides (CB1 e CB2). Exerce efeitos psicoativos, digestivos, emocionais e moduladores da dor. Já o CBD possui baixa afinidade por estes receptores e apresenta propriedades anti-inflamatórias, analgésicas, neuroprotetoras, anticonvulsivantes, antieméticas, antipsicóticas, dentre outras. Assim, canabinoides têm sido considerados para o tratamento de várias doenças neurológicas crônicas de difícil tratamento tais como a epilepsia refratária, doença de Alzheimer, doença de Parkinson, doença de Crohn, diabetes e câncer. Esta classe de compostos tem sido administrada principalmente pela via oral (uso farmacológico) ou através da vaporização (uso recreativo). Embora efetivas, estas moléculas apresentam alta lipofilicidade (log P entre 6 e 7), o que implica em uma série de desafios biofarmacêuticos que devem ser contornados, a considerar: baixa solubilidade aquosa, baixa absorção (20 a 30%) e biodisponibilidade oral (13 a 19%), demora para alcançar as concentrações plasmáticas terapêuticas devido à alta interação com membranas e elevado metabolismo hepático. Um maior depósito destes compostos no tecido adiposo também é observado, o que leva a resultados farmacocinéticos variáveis. Por fim, canabinoides são suscetíveis à degradação devido à ação da luz, temperatura e auto-oxidação. Assim, estudos com rotas alternativas de administração ou proposição de novas formulações tem sido considerados. Sistemas nanoparticulados tem se mostrado promissores, pois além de contornar problemas de solubilidade e estabilidade, podem incrementar a eficácia, reduzir efeitos adversos, permitir uma liberação sustentada e/ou um direcionamento sítio-específico. Face ao exposto, este trabalho de revisão narrativa traz uma oportunidade de discussão de vantagens e desvantagens de cada sistema nanoparticulado, permitindo identificar o tipo de nanopartícula mais apropriado para cada aplicação, além de tornar o desenvolvimento de formulações mais assertivo. Estudos publicados nos últimos 30 anos em revistas indexadas nas bases de dados *Scopus e Pubmed* foram considerados nesta análise. Os sistemas nanoparticulados foram agrupados em três categorias principais: sistemas

coloidais lipídicos (lipossomas, sistemas autonanoemulsionáveis (SNEDDS), nanoemulsões, carreadores lipídicos nanoestruturados e nanocápsulas lipídicas), sistemas coloidais poliméricos (micelas poliméricas e nanopartículas poliméricas) e sistemas coloidais inorgânicos (nanotubos de carbono e nanopartículas metálicas). A maior parte dos estudos considerou sistemas nanoparticulados lipídicos e poliméricos. Além de uma alta biocompatibilidade, sistemas lipídicos são caracterizados por proporcionar melhorias significativas na solubilidade e biodisponibilidade. SNEDDS, por exemplo, atravessam a barreira intestinal de forma efetiva devido ao reduzido tamanho (~30 nm), com um rápido alcance da concentração plasmática máxima, o que resulta em maior biodisponibilidade oral. Uma absorção pelo sistema linfático é observada quando estes sistemas apresentam lipídeos de cadeia longa em sua composição. Inibidores de metabolismo podem também ser incluídos para prolongar o tempo de ação. Sistemas lipídicos de THC tem também sido aplicados topicamente para o tratamento de glaucoma, com a obtenção de resultados de eficácia superiores a medicamentos já utilizados clinicamente. Sistemas poliméricos tem como principais diferenciais o fato de permitem um maior controle de liberação do ativo, maior estabilidade e a possibilidade de funcionalização de superfície (liberação sítio-específica), a qual torna a ação mais seletiva. Nos estudos analisados, o PLGA foi o copolímero mais utilizado, com a obtenção de uma liberação sustentada por vários dias. A liberação sítio-específica é particularmente relevante no tratamento de doenças do SNC e câncer já que as terapias atuais são caracterizadas por vários efeitos adversos. Micelas poliméricas de canabinóides tem se mostrado promissoras em terapias tumorais pois o reduzido tamanho permite acessar sítios tumorais mais facilmente devido ao conhecido efeito EPR. Além das vantagens decorrentes do reduzido tamanho, a modulação da carga de superfície destas partículas pode conferir propriedades adicionais. Sistemas carregados positivamente e administrados na mucosa nasal, por exemplo, podem ter sua retenção aumentada nesta região em função de interação eletrostática com a mucina, que é carregada negativamente. Ainda que as nanopartículas tenham demonstrado um papel promissor no tratamento de várias doenças difíceis de tratar, estudos translacionais devem ser intensificados para confirmar todos os benefícios aqui descritos.

Palavras-chaves: nanopartículas; canabinoides; sistemas coloidais lipídicos; sistemas coloidais poliméricos.

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ABSTRACT

The discovery of the endocannabinoid system in the 20th century has contributed to a growth in the number of studies with compounds affecting CB1 and CB2 receptors, which are highly expressed in Central Nervous System (CNS) and cancer cells. Flexibilization in the legislation related to the use of *Cannabis* in many countries has also contributed to a rapid introduction of new products in the market. On the other hand, these medicines often show biopharmaceutical limitations, particularly after an oral administration. In fact, cannabinoids are highly lipophilic molecules (*i.e.*, low aqueous solubility), which challenges the development of formulations and the transport of the active ingredients across biological membranes. These molecules are also susceptible to photo- and thermal degradation as well as auto-oxidation reactions. In this context, nanotechnology appears as a strategy to overcome such technological limitations. In addition to improving the solubility and absorption of cannabinoids, these systems may provide a sustained and/or site-specific delivery with greater efficacy and stability, and fewer adverse effects. In this review, the main types of cannabinoid-based nanoparticles (NPs) reported so far are addressed on the basis of the advantages and disadvantages of each system. Surface modification approaches of NPs, which allow to achieve a more selective action in CNS-affecting diseases (*e.g.*, multiple sclerosis) and cancer, are also presented. Formulation, preclinical and clinical studies performed with colloidal carriers were individually analyzed. Overall, this study aims at identifying promising systems for targeted applications to make future formulation optimization processes more effective and faster.

Keywords: nanoparticles; cannabinoids; lipid-based nanoparticles; polymer-based nanoparticles.

ABBREVIATION LIST

AUC area under the blood concentration *versus* time curve

BAAm N,N′-methylenebisacrylamide

BBB blood-brain barrier

CB1 cannabinoid receptor type 1

CB2 cannabinoid receptor type 2

CB13 naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone

CBD cannabidiol

CNS central nervous system

CNT carbon nanotubes

CS chitosan

CUR curcumin

CYP3A4 cytochrome P450 Family 3 Subfamily A Member 4

CYP2C19 cytochrome P450 Family 2 Subfamily C Member 19

DL drug loading

DMSO dimethyl sulfoxide

DRX x-ray diffraction

EDAC *N*-Ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide hydrochloride

EE encapsulation efficiency

EPR enhanced permeability and retention

FDA Food and Drug Administration

FTIR Fourier-transform infrared spectroscopy

GML glyceryl monolinoleate

GI or GIT gastrointestinal tract

IOP intraocular pressure

LCT long chain triglycerides

MBC minimum bactericidal concentration

MCT medium chain triglycerides

MIC minimum inhibitory concentration

MWCNT multiple layers of graphene sheets

NE nanoemulsion

NLC nanostructured lipid carriers

NP nanoparticle

PDI polydispersity index

PEG polyethyleneglycol

PEGDA poly(ethylene glycol) diacrylate

PLGA poly(lactic-co-glycolic acid)

Poloxamer 407 PEO_{101}-*b*-PPO_{56}-*b*-PEO_{101}

PNL pro-nanolipospheres

SCT short chain triglycerides

SMA poly(styrene)-co-maleic anhydride

SNEDDS self-nanoemulsifying drug delivery systems

SO sesame oil

SWCNT single layers of graphene sheets

Tf transferrin

THC tetrahydrocannabinol

TNBC triple-negative breast cancer

VHS valine-hemisuccinate

WIN WIN55,212-2

2-AG 2-arachidonoyglycerol

1. Introduction

The plant of *Cannabis sativa* presents in its composition more than a hundred phytocannabinoid compounds, including the cannabinoids Δ^9-tetrahydrocannabinol (Δ^9-THC), which is a psychoactive compound, and the nonpsychoactive cannabidiol (CBD) [1] (Fig.1). Δ^9-THC is a partial agonist of cannabinoid receptors (CB1 and CB2), which exerts psychoactive and pain modulatory effects via CB1 agonism. CBD, in turn, presents relatively low affinity for the orthostatic sites of these receptors; however, it can inhibit Δ^9-THC binding at CB1 receptors via a different mechanism [2]. CB1 receptors are mainly located in the central nervous system (CNS) whereas CB2 receptors are predominantly expressed in immune system tissues [3].

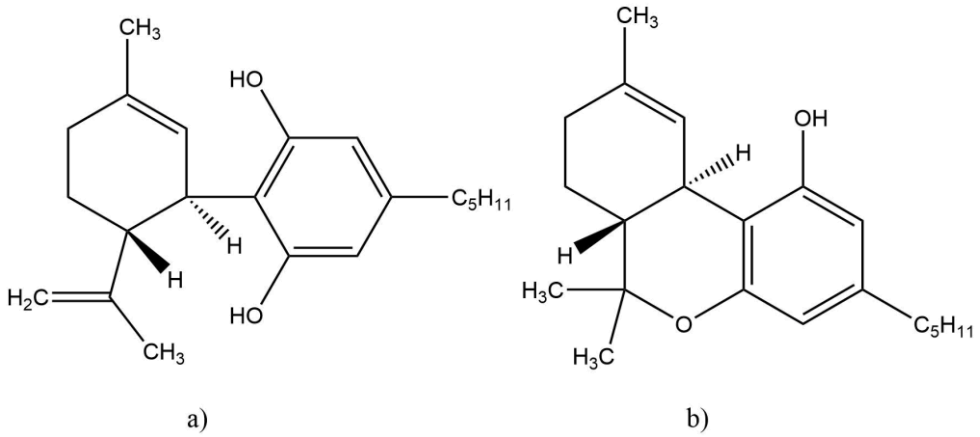

Fig. 1. Chemical structure of (a) cannabidiol and (b) Δ9-tetrahydrocannabinol.

In addition to a favorable safety and tolerability profile in humans [4], CBD has shown analgesic [5], neuroprotective [6], anticonvulsant [3,7], antiemetic [8], antispasmodic [9] and anti-inflammatory properties [3,10]. Its neuroprotective effect is particularly associated with the antioxidant and anti-inflammatory activity as well as the modulation of multiple brain biological targets. This is the reason why CBD has been considered for the treatment of various difficult-to-treat neurological diseases such as epilepsy, Alzheimer's disease and Parkinson's disease [11].

Different cannabinoid-based medicines are currently available on the world market. Sativex® (nabiximol) was the first cannabis-based product marketed in UK and other European countries. It is indicated to treat spasticity in patients affected by multiple sclerosis and is commercially available as an oromucosal spray that contains an equimolar mixture of CBD and Δ^9-THC obtained from the ethanolic extract of cannabis [12]. Epidiolex®, a CBD-only oral solution, is approved by the US FDA for the treatment of epileptic seizures, Lennox-Gastaut

and Dravet syndrome in children 2 years and older [13]. Synthetic Δ^9-THC alone has been commercialized as both oral capsules (Marinol®, dronabinol) and oral solution (Syndros®). Both dosage forms are used for the treatment of cancer chemotherapy-induced nausea and anorexia associated with weight loss in patients with AIDS [14]. Additionally, Cesamet®, a medicine composed of a synthetic analogue of Δ^9-THC known as nabilone, is approved to treat neuropathic chemotherapy-induced nausea [15].

The increasingly number of cannabis-based products and their medical applications opens new opportunities for advanced routes of administration and delivery systems [16]. Cannabis products are commonly either inhaled by smoking/vaporization or taken orally. Although the oromucosal, transdermal and rectal routes are not widely used, they show the advantage of avoiding the liver first-pass effect [17]. This is particularly relevant for formulations with CBD because it suffers extensive first-pass metabolism [18]. CBD undergoes monohydroxylation at C-7 forming the 7-OH metabolite, a process mediated by CYP3A4 and CYP2C19 [19]. Another prominent metabolic route of CBD is the direct glucuronidation of the parent compound, leading to the formation of an O-glucuronide [20]. The metabolites are preferentially excreted via the kidneys [18].

The absorption of cannabinoids varies from 20-30% for oral administration and up to 10-60% for inhalation [21]. As cannabinoids have a log P > 5 and solubility higher than 50 mg/g in long-chain triglyceride carriers, they can also be orally absorbed through intestinal lymph capillaries by associating with lipoproteins known as chylomicrons [22,23]. After disposition in bloodstream, a preferential accumulation in fatty tissues is observed in these compounds [24], which explain the gradual release from these tissues, prolonged elimination half-life and variable pharmacokinetic profiles [25].

In addition to high lipophilicity (log P = 6-7), cannabinoids present very low aqueous solubility (2-10 μg/mL) [21], and are susceptible to degradation due to action of light or temperature, and suffer auto-oxidation [26]. These characteristics make them interesting candidates for nano-based formulations. Nanotechnology can be used to improve both the solubility and the physicochemical stability of cannabinoids. The increased efficacy and bioavailability, toxicity reduction, controlled release, and targeted delivery that could be achieved by considering the application of such systems in formulations containing cannabinoids are highly attractive [16]. In this review, the main nano-based technologies that have been reported in the cannabinoid literature are presented (Table 1).

Table 1. Advantages and disadvantages of nano-based technologies for cannabinoids.

NANOPARTICLE TYPE	ADVANTAGES	DISADVANTAGES
POLYMER-BASED COLLOIDAL SUSPENSIONS		
Polymer micelles	-Increased solubility for cannabinoids -Drug release in controlled manner -Prevents psychotropic effects of cannabinoids (these NPs do not cross the blood-brain barrier) -EPR effect due to small particle size (desirable in antitumor therapies)	-Low drug-loading efficiency -Low physical stability *in vivo* -Dependency of critical micelle concentration
Polymer nanoparticles	- Drug release in controlled manner (several days), which is particularly interesting in chronic therapies -Increased stability and protection against degradation compared to lipid systems -Site-specific drug delivery -Drug release profile can be adjusted depending on polymer type -Preparation methods are versatile	-Polymer degradation products can be toxic -Preparation methods usually use toxic organic solvents
LIPID-BASED COLLOIDAL SUSPENSIONS		
Liposomes	-The amount of cannabinoid released can be modulated by the phospholipid/cholesterol ratio in the membrane -Capacity for self-assembly -High biocompatibility and interaction with biological membranes -Promising systems for topical release of cannabinoids (skin/eye)	-Lower encapsulation efficiency of cannabinoids than other NPs -Low stability in biological fluids -Rapid tissue distribution or short half-life (not appropriate for sustained drug release) -Phospholipid may undergo oxidation and hydrolysis-like reactions -High production cost -The traditional preparation method uses organic solvents -May trigger immune response
Self-nanoemulsifying drug delivery systems	-Ease of preparation and scale-up -Low production cost -Provide high oral bioavailability -Reduced particle size -Reduce the effect of bile salts on cannabinoid absorption -Shorter onset of action (reduce T_{max})	-Wide particle size range as nanoparticles are formed *in situ* -Precipitation of drug in gastrointestinal fluid is more common than other lipid-based systems
Nanoemulsions	-More stable systems than liposomes -Enhanced drug solubility and bioavailability -Improved hydration when used topically -Low production cost -High encapsulation efficiency for cannabinoids	-Instability phenomena such as coalescence, oxidation of oils or lipids used in the formulation can be observed
Nanostructured lipid carriers	-Ease of large-scale production using high pressure homogenization technique	-These particles are more sensitive to changes in preparation or

	-Imperfections in the lipid matrix accommodates cannabinoids more easily than solid lipid nanoparticles, impacting on encapsulation efficiency -Prevent the particle from coalescing due to the solid matrix compared to nanoemulsions	storage parameters, which can lead to lipid phase transitions -Although these systems are more stable than solid lipid nanoparticles, long-term stability issues upon storage are still observed
Lipid nanocapsules	-Lipid core increases cannabinoid incorporation rate whereas the polymer coating provides more stability -Efficient surface functionalization due to the presence of the polymer -Low energy methods may be used in preparation without using high amount of surfactant and co-surfactant	
INORGANIC COLLOIDAL SUSPENSIONS		
Carbon nanotubes	-Improved mechanical properties (high tensile strength) -Penetrate cell membranes due to reduced particle size -Intrinsic spectroscopic properties (e.g., Raman and photoluminescence) allow a tracking and real-time monitoring of drug performance	-Expensive production -Low degradability -Toxicological issues (pulmonary complications)
Metal nanoparticles	-Specific optical and magnetic properties (important for cancer applications) -Strong plasma absorption -Multimodal applications -Particles are usually characterized by a uniform size and shape	-Toxicological issues -Impurities from the synthesis of these nanoparticles can result in unstable systems (NPs are very reactive).

2. Lipid-based colloidal systems

2.1. Liposomes

Liposomes are small spherical-shaped vesicles that can be prepared from cholesterol and phospholipids. They consist of one or more phospholipid bilayer membranes surrounding aqueous units. The selection of the type of bilayer constituents determines the ‘rigidity’ or the ‘fluidity’ of the system, as well as the charge of the bilayer. Unsaturated phosphatidylcholines, for instance, result in much more permeable and less stable bilayers whereas saturated phospholipids with long acyl chains show the opposite effect [27]. Different cannabinoids also interact differently with lipid bilayers of liposomes. Studies with spin-labeled lecithin/cholesterol liposomes showed that cannabinol and cannabidiol increase the molecular order of the liposomal bilayer, making its structure more rigid. An opposite effect was observed

for psychoactive cannabinoids as Δ^9-THC. A similar behaviour is expected in lipid bilayers of biological membranes [28]. Although liposomes represent one of the most studied drug delivery systems and has been used in several commercially approved products; they show some disadvantages as carrier/vehicles for cannabinoids, mainly the low EE that results from the limited ability to hold and stabilize such compounds within the phospholipid bilayer [17].

In an *in vivo* assay with Δ^9-THC-loaded liposomes, Hung et al. (2001) found rapid bioavailability and early onset of pharmacological effect after pulmonary administration (approximately 10-20 % of the total Δ^9-THC dose). The formulation was composed of different molar ratios of dipalmitoyl phosphatidylcholine and cholesterol (9:1, 7:3 or 6:4). A particle size range 300-400 nm was achieved for these combinations, which presented a Δ^9-THC content of 300 mg/mL (E.E. data were not included in this patent). The use of high proportion of cholesterol in the system was crucial to control the pharmacokinetic of the liposomes by a mechanism that involves the phospholipid membrane rigidity. The dipalmitoyl phosphatidylcholine: cholesterol composition in a molar ratio of 7:3 was systemically released over a longer period compared to formulations with a lower relative amount of cholesterol. Although a rapid onset of action was found for the formulation, the therapeutic effects lasted more than 24 h after pulmonary administration (with the Δ^9-THC release of 80-90%) and it was not affected by hepatic first-pass elimination [29].

Δ^9-THC-loaded liposomes delivered intratracheally and intraperitoneally have also been considered for intraocular pressure (IOP) reduction in studies performed in Brown Norway rats. The ocular hypotensive activity of cannabinoids has been attributed to the activation of CB1 receptors localized to the anterior structures of the eye. Liposomes composed of dipalmitoyl phosphatidylcholine and cholesterol (7/3) were prepared by the extrusion method and presented a mean particle size of 368 nm and E.E. values greater than 90%. Intratracheal delivery of Δ^9-THC-loaded liposomes resulted in a faster reduction of IOP than intraperitoneal administration, which was associated to the rapid absorption of the Δ^9-THC from the alveoli into the systemic circulation. Although the maximal efficacy was comparable between both administration routes, intratracheal delivery resulted in a lower ED_{50} (the dose of agonist producing 50% maximal change in IOP) or higher potency. The ED_{50} values were 0.08 and 0.12 mg/kg for intratracheal and intraperitoneal of Δ^9-THC-loaded liposomes, respectively. A short duration of the Δ^9-THC effect was found in both situations, which was explained by the rapid redistribution of this lipophilic compound to other tissues. The authors suggested that

PEGylation of these delivery systems could be considered in future studies aiming to extend their circulation time [30].

Liposomes were also used to improve the oral bioavailability of CBD in osteoarthritis treatment models. Firstly, the authors confirmed the already known anti-inflammatory activity of the CBD in both *in vivo* and *in vitro* models, demonstrating substantial impact on inflammatory cytokines and innate immune cell subsets associated with pathophysiology of arthritis. CBD-loaded liposomes were then prepared by using a sunflower lecithin (phosphatidyl choline) base. Each liposome had a particle size of approximately 100 nm and 10-20 mg/mL CBD. These nanocarriers showed to be stable at room temperature and 4 °C and between pH 5-9 for 3 months. In assays with healthy human volunteers, the oral bioavailability of CBD from liposomes was about 17-fold greater than that of free CBD at one-hour post administration. Twenty dogs diagnosed with osteoarthritis were considered for activity evaluation. These animals randomly received placebo, 20 or 50 mg/day CBD and 20 mg/day CBD-loaded liposome. Clinical examination using the Helsinki Chronic Pain Index (HCPI) were performed on study days 0, 30 and 45. Significant improvements in quality-of-life scores (sitting to standing, lying to standing, walking and running) were observed only in animals who received 50 mg/day CBD and 20 mg/day liposomal CBD. The CBD encapsulation was crucial to increase its activity when low dose of this agent was considered. This compound seems to act by reducing proinflammatory cytokines and pathologic neutrophil activity [31].

2.2 Self-nanoemulsifying drug delivery systems

Lipid-based self-emulsifying drug delivery systems are homogenous mixtures of an active lipophilic compound with lipids, surfactants and co-solvents. In the upper gastrointestinal lumen, these agents spontaneously lead to the formation of O/W nanoemulsions, which may enhance both solubility and oral absorption of molecules [32] (Fig. 2; Table 2). Due to the small size (approximately 30 nm), these particles are able to spread over a large surface area and access the intervillous spaces of the intestinal brush border [33]. The surfactant in the formulations minimize drug precipitation phenomena, which also facilitates absorption [34].

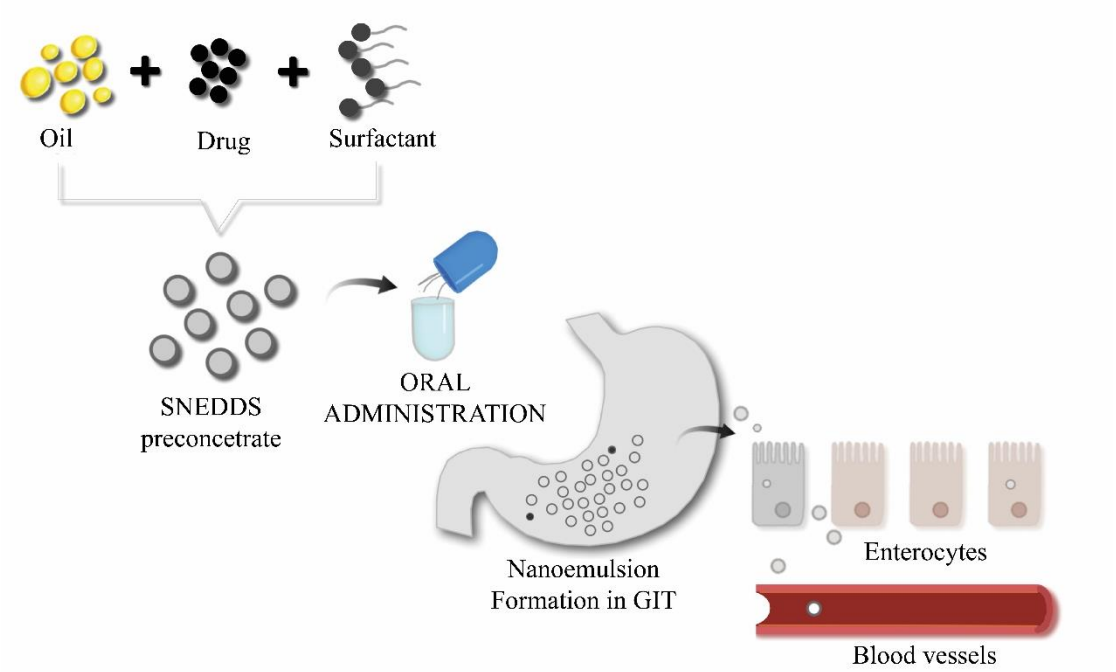

Fig. 2. The process of encapsulation, nanoemulsion formation and absorption of self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of cannabinoids. The oil, drug, and surfactant initial mixture (known as "preconcentrate") rapidly form nanoemulsions under gastrointestinal tract motility. The size of this nanoparticles allows to access intestinal intervillous spaces and finally, the blood vessels.

The type and length of the lipid chain, degree of saturation, and digestibility of the oily excipients from these systems may affect the oral absorption of lipophilic drugs [23]. Overall, short- and medium-chain triglycerides (MCT) reach the systemic circulation through the portal system. Long-chain triglycerides, in turn, are absorbed via the intestinal lymphatic pathway through association with lipoproteins, bypassing the liver first-pass metabolism [22,35].

Kok et al. (2022) developed self-nanoemulsifying drug delivery systems (SNEDDS) aiming to improve the biopharmaceutical properties of CBD. After a solubility screening, Brij O10 (surfactant), Captex 355 (oil carrier) and propylene glycol (co-solvent) were selected as formulation constituents. *In vitro* dispersion studies did not show any CBD precipitation. In digestion assays performed *in vitro*, approximately 80% of the initial concentration of CBD remained solubilized by the SNEDDS. A formulation containing medium chain triglyceride (MCT-CBD) and another sesame oil-based formulation with similar composition to Epidiolex® (SO-CBD) was also considered for comparison. Unlike the SNEDDS, both control formulations did not disperse properly in the biorelevant media. All formulations were administered to healthy female Sprague-Dawley rats via oral gavage (20 mg/kg CBD) for pharmacokinetic studies. CBD-SNEDDS (particle size of 48 and 175 nm) provided a faster absorption of CBD than MCT-CBD (median T_{max} values: 0.5-1 h versus 6 h). AUC values showed a greater exposure to CBD from the CBD-SNEDDS compared to MCT-CBD. The improved

solubilization of CBD in the GI fluids provided by the SNEDDS could explain these findings. On the other hand, SO-CBD formulation resulted in a greater AUC compared to SNEDDS. The C_{max} values, in turn, suggested that all formulations achieved similar maximum plasma concentrations of CBD. No *in vitro-in vivo* correlation was observed in this study [36].

Nakano et al. (2019) have also performed pharmacokinetic studies with CBD-SNEDDS in rats; however, they also evaluated the effect of bile secretion on the intestinal absorption in a bile-fistulated rat model. The optimal proportion of SNEDDS constituents (vitamin E acetate, ethanol, Tween® 20 and distilled water) was defined based on a ternary phase diagram and resulted in particles with a size of 35.3 nm. Interestingly, CBD-SNEDDS shortened the mean T_{max} by approximately 3 times and increased the $AUC_{0-\infty}$/dose (oral bioavailability) by 65% compared to CBD oil. In rats with biliary fistulas treated orally only with CBD oil, $AUC_{0-\infty}$/dose and C_{max}/dose reduced by a factor of 27 and 23, respectively. In contrast, no significant changes in these pharmacokinetic parameters were observed for CBD-SNEDDS in the comparison between the bile-fistulated and untreated rats. These last findings suggest that the absorption of CBD oil depend on the bile-mediated micelle formation, which was not found for CBD-SNEDDS. Therefore, a less impact of food matrices on the absorption of CBD is expected from SNEDDS [37].

In addition to animal testing, the oral bioavailability of CBD from SNEDDS was tested in human healthy volunteers under fasted conditions (*n*=16). SNEDDS formulations based on the VESIsorb® technology (emulsifiers, edible vegetable oils and fatty acids; droplet size of 40-50 nm) were developed and loaded with Hemp-extract (25 mg CBD). This plant extract is characterized by a CBD content of 60% and Δ^9-THC concentrations lower than 0.05%. The same concentration of Hemp-extract dissolved in medium-chain triglycerides (MCT-CBD) was used as reference formulation. Single oral administration of SNEDDS-CBD increased the C_{max} and AUC_{0-8h}/AUC_{0-24h} in 4.4-fold and 2.85-/1.70-fold compared to the reference formulation, respectively. As already observed in animal assays, T_{max} was significantly shorter for SNEDDS-CBD compared to MCT-CBD (1 *vs.* 3 h). The SNEDDS also reduced the coefficient of variation, indicating a decreased interindividual variability [38].

De Prá et al. (2020) evaluated the effect of different lipid vehicles on the oral absorption of CBD in animal and human pharmacokinetic models. Medium-chain triglycerides (MCT), glyceryl monolinoleate (GML) and SNEDDS formulation were tested. In *in vitro* digestion assays, SNEDDS yielded the highest CBD recovery in the aqueous phase, followed by GML and MCT (86, 13 and 5.6%, respectively). In mice, SNEDDS (particle size of 148 nm and zeta

potential of -9.5 mV) increased the AUC_{0-6h} of CBD in 1.48 and 3.97 times compared to GML and MCT formulation, respectively. In a single-dose, open-label, crossover study performed in 11 volunteers, SNEDDS (particle size of 194 nm and zeta potential of -6.9 mV) increased the AUC_{0-12h} of CBD by 1.12 and 1.48 times compared to GML and MCT, respectively. Taken together, these results show that the oral bioavailability of cannabinoids is affected by the physicochemical characteristics of lipids, the length of the fatty acid chain and the susceptibility to digestion. In addition to specific solubilization mechanisms, the GML formulation could improve the CBD bioavailability through intestinal lymph capillaries as it is composed of long-chain triglycerides. No correlation between the *in vitro* digestion assays and *in vivo* assays was observed [22].

Formulations based on pro-nano dispersion technology have also been tested clinically (named herein as PTL401). The system composition included Δ^9-THC, CBD, polysorbate 20, sorbitan monooleate 80, polyoxyethylene hydrogenated castor oil 40, tricaprine, lecithin and ethyl lactate. These formulations were obtained by a pre-concentration method, displaying a particle size of 30 nm and PDI between 0.1 and 0.2. The study included 15 healthy volunteers treated with both formulations, considering a washout period of 7 days between applications. Each volunteer received a single dose of three PTL401-filled capsules (Δ^9-THC/CBD = 10.8/10 mg) and an equivalent dose of the oromucosal spray Sativex®. PTL401 provided a significantly shorter T_{max} than the reference spray. C_{max} of both Δ^9-THC and CBD from PTL401 was approximately 1.6-fold higher than the Sativex®. The relative bioavailability of CBD and Δ^9-THC, which was calculated from the AUC ratios, was 31% and 16% higher than reference formulation [33].

Advanced SNEDDS named “pro-nanolipospheres” (PNL) including absorption chemical enhancers and metabolism inhibitors have also been suggested in recent studies aiming to improve the biopharmaceutical properties of cannabinoids [39,40]. For example, a combined administration of Δ^9-THC and CBD was advantageous and undesirable effects such as anxiety, panic, sedation, dysphonia and tachycardia were minimized. Studies with different PNL were initially performed in animals but followed by clinical trials after formulation optimization. Also, piperine, curcumin and resveratrol were individually included into the formulation as inhibitor of phase I and phase II metabolism enzymes. CBD-PNLs were prepared by a preconcentrate method. Initially, soy lecithin and an amphiphilic co-solvent are mixed at 40 ^{o}C. Then, triglyceride (tricaprin), polyoxyl 40-hydroxy castor oil, Tween® 20, and Span® 80 were added and homogenized at the same temperature. After the formation of a homogenous

mixture, 3% (w/w) of CBD was added. The CBD-piperine-PNLs presented a 30 nm average diameter, -15 mV surface charge and 0.23 PDI. CBD-resveratrol-PNLs, in turn, presented a 65 nm average diameter, -10 mV surface charge and 0.5 PDI. For PNLs containing curcumin, stability problems or CBD precipitation events were observed. As already mentioned, the nanometric size is crucial to penetrate the inter-villous spaces at the intestinal brush border (50-250 nm). The addition of resveratrol into PNLs did not affect the bioavailability of CBD significantly. On the other hand, a single oral administration of CBD-piperine-PNL to rats increased in approximately 2- and 6-fold the AUC when compared to CBD-PNL (without piperine) and CBD solution, respectively. When Δ^9-THC was incorporated into the piperine-PNL, its oral bioavailability increased 9.3-fold in relation to Δ^9-THC solution. Taken together, these results suggested that the inclusion of piperine into PNLs is effective in improving the oral bioavailability of cannabinoids due to inhibitory effect on phase I and phase II metabolism enzymes as well as intestinal efflux mechanisms [40].

In view of these promising results, a clinical study comparing the performance of Δ^9-THC/CBD-piperine-PNL with a marketed spray (same active compounds) was carried out. The study included 9 healthy subjects under fasted conditions. Each volunteer received a Δ^9-THC/CBD (10.8 mg, 10 mg; respectively) piperine (20 mg)-PNL filled capsule and an equivalent dose of the oromucosal spray Sativex®. A washout period of 21 days was considered between the treatments. PDI, particle size and zeta potential were 0.23, 40 nm and -12.5 mV, respectively. Single oral administration of the piperine-PNL formulation provided a 3-fold increase in C_{max} and a 1.5-fold increase in AUC for Δ^9-THC when compared to Sativex®. For CBD, a 4- and 2.2-fold increase in C_{max} and AUC was found, respectively. No serious adverse events, severe cardiovascular or intoxication effects were observed during this study [39].

Table 2. Impact of cannabinoid incorporation in self-nanoemulsifying drug delivery systems on pharmacokinetic.

SNEEDS composition	Tested material	Particle size (nm)	Pharmacokinetic model	Pharmacokinetic data	Reference
Captex 355, Brij O10, Propylene glycol	CBD	48 and 175 nm	Rats	SNEDDS resulted in a faster absorption (shorter T_{max}) than a formulation containing medium chain triglycerides; however, an AUC value similar to a formulation composed of sesame oil (similar composition to Epidiolex®)	Kok et al. (2022)
Vitamin E acetate, Tween® 20	CBD	35.3 nm	Normal and bile-fistulated rats	>SNEDDS shortened Tmax by about 3 times and increased the $AUC_{0-\infty}$/dose by 65% compared to CBD oil. >Oral absorption of CBD showed to be mediated by biliary micelles.	Nakano et al. (2019)
VESIsorb® technology (emulsifiers, edible vegetable oils and fatty acids)	Hemp-extract (25 mg CBD)	40-50 nm	Human healthy volunteers under fasted conditions	>SNEDDS increased the C_{max} and AUC_{0-8h}/AUC_{0-24h} in 4.4-fold and 2.85-/1.70-fold compared to the reference formulation (extract dissolved in medium-chain triglycerides). >Shorter T_{max} for SNEDDS (1 vs. 3h)	Knaub et al. (2019)
Glyceryl monolinoleate, polyoxyl 40 castor oil, polyethylene glycol 400	CBD	148 and 194 nm	Mice and human volunteers	>In both models, SNEDDS increased the AUC compared to glyceryl monolinoleate (GML) and medium chain triglycerides (MCT) formulation (1.12-3.97 times). >GML could improve CBD absorption through intestinal lymph capillaries.	De Prá et al. (2021)
Polysorbate 20, sorbitan monooleate 80, polyoxyethylene hydrogenated castor oil 40, tricaprine, lecithin and ethyl lactate	Δ^9-THC/ CBD	30 nm	Healthy human volunteers	>SNEDDS resulted in a shorter T_{max} value than reference formulation (Sativex®) >Bioavailability of CBD and Δ^9-THC was 31 and 16% higher than market formulation	Atsmon et al. (2018)
Soy lecithin, ethyl lactate, tricaprin, polyoxyl 40-hydroxy castor oil, Tween® 20, and Span® 80	Δ^9-THC and CBD	30 nm	Rats	>SNEDDS with CBD and piperine (metabolism inhibitor) increased in about 2 and 6-fold the AUC compared SNEDDS without piperine and CBD solution. >SNEDDS with Δ^9-THC and piperine increased the bioavailability in 9.3-fold compared to Δ^9-THC solution	Cherniakov et al. (2017b)

Soy lecithin, ethyl lactate, tricaprin, polyoxyl 40-hydroxy castor oil, Tween® 20, and Span® 80	Δ^9-THC/ CBD	40 nm	Healthy human volunteers	>SNEDDS increased the AUC value of Δ^9-THC and CBD in 1.5- and 2.2-fold compared to Sativex®. >SNEDDS increased C_{max} of Δ^9-THC and CBD in 3 and 4-fold compared to Sativex®.	Cherniakov et al. (2017a)

2.3 Nanostructured lipid carriers

Nanostructured lipid carriers (NLCs) are a second generation of lipid nanoparticles developed to overcome the shortcomings of first-generation solid lipid nanoparticles. Biodegradable and compatible lipids (solid and liquid) and emulsifiers are combined for the preparation of NLCs. Liquid lipids result in structural imperfections or less ordered crystalline arrangement, which improve drug loading and reduce drug expulsion during the storage [41]. Different cannabinoids (URB597, AM251 and rimonabant) were incorporated into NLCs aiming to improve solubility and encapsulation efficiency. AM251 and rimonabant are inverse agonists of the cannabinoid receptor CB1 while URB597 is a fatty acid amide hydrolase inhibitor. NLCs were prepared by a method based on lipid melting and ultrasonication. A direct and an inverse preparation method were considered to improve the encapsulation rate. In the first method, the oil phase (OP) was added to the water phase (WP). In the inverse preparation method, the order of phase mixing was reversed. The OP was composed of a lipid mixture of tristearin/miglyol 2:1 (w/w) whereas the WP was composed of an aqueous poloxamer® 188 solution. The direct preparation method resulted in particles with low recovery or E.E of active compounds due to instability phenomena (coalescence, lipid aggregation and lipid adhesion in the glassware wall). On the other hand, the inverse preparation method resulted in more stable nanoparticles, which showed high E.E. (higher than 92%) and a mean particle diameter close to 100 nm. E.E. values of 99.9% were achieved for NLCs containing AM251. For rimonabant-NLCs, E.E. values of 98% were found by using the inverse preparation method, a 30% increase compared to direct method. In summary, the study indicated that changes in the NLC preparation protocol led to increased cannabinoid encapsulation rates, resulting in formulations suitable for *in vitro* and *in vivo* clinical and preclinical studies [42].

Given that the nasal route can prevent liver metabolism and increase brain bioavailability, mucoadhesive NLCs were developed for nasal delivery of CBD aiming to treat neuropathic pain. NLCs were prepared by the hot microemulsion technique combining stearic acid and oleic acid as solid and liquid lipid, respectively. Cetylpyridinium chloride was included

to generate particles with a positively charged surface and Span 20® as a co-surfactant. The particle size, zeta potential, PDI and E.E of CBD-NLCs was 177 nm, +41 mV, 0.3 and 99.99%, respectively. DRX and thermal analyses suggest that CBD is found in an amorphous state in these nanoparticles. FTIR analyses do not indicate a CBD distribution on the particle surface. A biphasic release pattern was identified in *in vitro* release assays performed in phosphate buffer (pH 6.8) containing 5% (w/v) Tween 80. After an initial burst (> 50% of CBD released within 5 min), a slow and sustained release was observed. Interactions of NLCs with negatively charged mucin explain the found mucoadhesion (Fig. 3). In *in vivo* assays, the nasal administration of CBD-NLC dispersion resulted in a more significant and lasting antinociceptive effect in male Swiss mice with neuropathic pain than the oral or nasal administration of CBD solution. The results found in this formulation show that the nasal route is more effective to transport CBD directly to the brain than the oral administration [43].

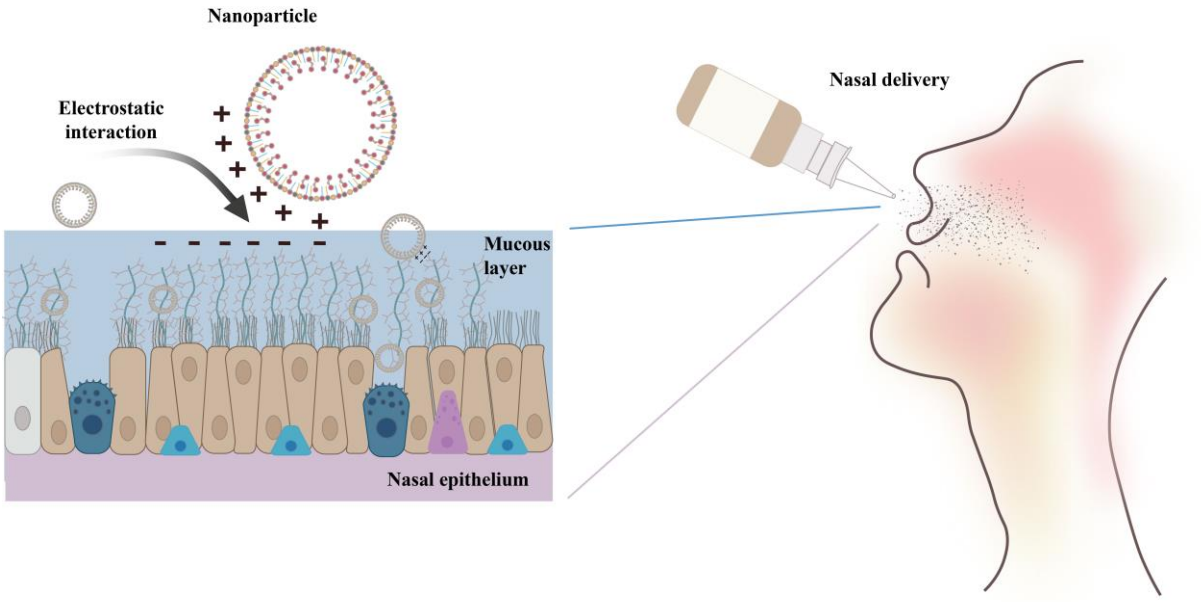

Fig. 3 Electrostatic interaction between positively charged nanoparticles and negatively charged mucin from the nasal mucosa.

NLCs were also developed to incorporate Δ^9-THC aiming to overcome its low aqueous solubility and high oxidation. Particles were developed by a hot high-pressure homogenization technique after mixing an aqueous phase composed of water and stabilizer in a lipid phase composed of cetyl palmitate (resulted in more stable nanoparticles compared to Compritol® 888) and Δ^9-THC. Once these systems were intended for nasal delivery, optimized NLCs (+/- 200 nm; > +45 mV) were stabilized with cetylpyridinium chloride to ensure the mucoadhesion

of particles. In fact, nanoparticles prepared with this cationic stabilizer agent showed higher mucoadhesiveness properties. In *in vitro* release study, more than half of the loaded Δ^9-THC was released after 15 min [43]. As lipophilic drugs are rapidly absorbed in nasal mucosa [44], the authors suggest that the high release rate of Δ^9-THC found for NCLs would facilitate the absorption. The solid matrix of the NLC provided a stabilizing effect on Δ^9-THC, where 91% of this compound was quantified after a 6-month storage at 4 °C for the most stable formulation. Under stress conditions at 40 °C, the compound amount was reduced to 79%. Interestingly, nanoparticles were nebulized from a commercial nasal spray bottle without any change in size. Taken together, these results suggest that the purposed system is promising for nasal delivery [43].

Finally, NLC has been considered for the incorporation of CB13, a cannabinoid that acts as a potent agonist of CB1/CB2 receptors. This compound is characterized by lower penetration rate into the brain than other cannabinoids, which result in less adverse effects. It is also characterized by a considerably low aqueous solubility (0.000134 mg/L) that impairs its drugability. NPs composed of either glyceryl dibehenate or glyceryl palmitostearate and stabilized with two different surfactants (polysorbate 20 and sodium deoxycholate) were prepared through the emulsification-solvent evaporation method. Lecithin was also considered as an additional emulsifier agent in some formulations. NPs with glyceryl palmitostearate in the lipid matrix were more stable at 4 °C and showed higher E.E. (equal or higher 89%) than NPs with glyceryl dibehenate. The higher hydrophobicity of glyceryl dibehenate increases the viscosity of the NP dispersion, resulting in more heterogeneous systems or higher PDI values. The solvent:lipid ratio was the main parameter that affected particle size. All NPs presented negative zeta potential values close to -30 mV. The optimal formulation was stable under simulated intestinal conditions and no cytotoxicity was found against NIH 3T3, HEK293T and Caco-2 cell was found after treatment with 250 mg/mL of each NP for 24 h. Activity assays with the optimal formulation were not performed [45].

2.4 Lipid nanocapsules

Lipid nanocapsules represent a recently developed nanocarrier type composed of a lipid/oily core surrounded by a surfactant shell. Among the advantages, they may provide improved bioavailability, increased drug targeting, and controlled drug release [46]. As the core of these particles is oily, cannabinoids can be efficiently incorporated [47,48].

CBD-functionalized lipid nanocapsules has been developed as innovative nanocarriers aiming to increase the transport rate of this active molecule through the blood-brain barrier (BBB). CBD has high affinity with various receptors located on the brain endothelium (e.g., CB_1, 5-HT_{1A}, TRPV-1, GPR55, D2), which contributes to an increased cell internalization rate. Monodisperse nanocapsules were prepared by the phase inversion temperature method and decorated with the nonpsychotropic cannabinoid CBD. NPs presenting different particle sizes (20-60 nm) were obtained by changing the constituent proportion (Labrafac lipophile WL 1349, Kolliphor HS15, Lipoid S75, NaCl and water) to investigate whether this parameter impacts on the transcytosis mechanisms. Both permeability assays in hCMEC/D3 cells and biodistribution studies showed that the highest brain transcytosis rate was achieved with the smallest CBD-decorated nanocapsules. A higher available plasma concentration and lower recognition by the reticuloendothelial system of these particles could explain these findings. These NP systems represent a promising platform for the design and development of novel therapies for CNS diseases. Further studies on the expression of receptors and transporters at the BBB are needed to measure the potential targeting mechanism [47].

In addition to surface functionalization with CBD, lipid nanocapsules were also loaded with CBD [48]. The activity of these systems against human glioblastoma cells was evaluated. CBD exhibits different biological actions on tumoral cells. It leads to apoptotic cancer cell death due to production of reactive oxygen species, impair tumor angiogenesis and reduce cell migration. The NPs were prepared by the same method described previously. E.E. and PDI values were higher than 94% and lower than <0.15, respectively. After surface functionalization, high adsorption of CBD on particle surface was also found, which was attributed to high affinity of lipophilic CBD by amphiphilic surfactant interface. Smaller nanocapsules showed to be more cytotoxic against U373MG cells (human glioblastoma cell line). The reduction of particle size from 50 to 20 nm reduced by 3.0-fold the IC_{50}. The surface functionalization of blank NPs with CBD also enhanced the *in vitro* glioma targeting properties by 3.4-fold compared to undecorated counterparts. The surface modification of CBD-loaded lipid nanocapsules with CBD, in turn, further reduced the IC_{50} values than undecorated counterparts. Taken together, these results suggest that a diverse distribution of active in the particle structure may contribute to both increased activity against glioma cells as transport through the BBB [48].

2.5 Nanoemulsions

Nanoemulsions (NEs) are isotropically dispersed systems of two non-miscible liquids (an oily and an aqueous phase), forming droplets or oily phases of nanometric size. NEs are more thermodynamically unstable systems than microemulsions due to the high energy required for the stabilization of surface and the formation of particles. Ostwald ripening, creaming, flocculation, and other physical instability events may be found, which require an appropriate concentration and combination of surfactants [49]. NEs may be used to improve both topical and oral bioavailability of cannabinoids [37,50].

CBD receptors are found in skin structures, which have been associated with disorders such as atopic dermatitis, itching, acne, hair growth, and hyper/hypopigmentation. Antioxidant and anti-inflammatory properties of CBD may also contribute to prevent skin ageing. As CBD-based oils and creams administered topically are characterized by a relatively low skin permeation/penetration rate, the development of NEs has been alternatively purposed. In a literature study, two methods were selected for NE preparation: sonication and two-stage high-pressure homogenization (microfluidization). Polyethylene glycol sorbitan monooleate and surfactin were used as surfactants for system stabilization. The high-pressure homogenization technique reduced the hydrodynamic radius and PDI to almost half when compared to the sonication method. The optimal NEs obtained by the microfluidization technique presented spherical shape and particle size close to 200 nm. The surfactant combination was crucial to maintain the pH of the formulation close to skin physiological range. NEs maintained stable particle size values within 30 days after a storage at 25 ^{o}C. The systems were not cytotoxic for human skin cell lines HaCaT keratinocytes and NHDF normal human dermal fibroblasts (cell viability greater than 80%). Finally, formulations showed a positive effect on skin hydration in assays with humans. Although cutaneous permeation studies with the developed formulation have not been performed, this increased skin hydration could facilitate the passive transport of cannabidiol to deeper layers of the skin [50].

Although the Δ^9-THC has been considered as a promising therapeutic agent for glaucoma, its low aqueous solubility and barrier properties of the transcorneal membrane limits the development of new topical delivery systems (e.g., eye drops). While prodrugs of this compound such as Δ^9-THC-valine-hemisuccinate (THC-VHS) have been suggested to solve solubility problems, their incorporation into NEs mainly improve the transcorneal transport. In this context, NEs were developed by homogenization technique followed by ultrasonication

and the effect of the optimal formulation on intraocular pressure (IOP) was compared to that of other systems (commercial timolol, and latanoprost ophthalmic solutions and emulsion in Tocrisolve™) in a nonpigmented normotensive NZW rabbit model [51]. NEs were composed of sesame oil, Tween® 80 and Poloxamer® 188 and presented an average diameter below 300 nm. This particle size is compatible with a penetration through the polarized epithelial cells. THC-VHS-NE resulted in a more significant reduction in IOP and a longer duration of action compared to the emulsion and both commercial formulations containing the VHS. The maximum reduction in IOP was 4.2, 2.6 and 2.4 mmHg for THC-VHS-NE, timolol and latanoprost, respectively. Timolol showed the shortest duration of action (150 min) whereas THC-VHS-NE formulation and latanoprost solutions had a similar duration of action (360 min). Filtration did not impact on NE attributes and thus it may be considered as a suitable sterilization method [51].

3. Polymer-based colloidal systems

3.1 Polymer micelles

Polymer micelles are composed of amphiphilic co-polymers self-assembling into spherical nanostructures consisting of a hydrophobic core and a hydrophilic corona. The main advantage of micelles over other nanoparticle types is the small particle size, an interesting asset for cancer application [52]. The low particle size allows the drug to reach the tumor region more easily through the mechanisms of enhanced permeability and retention (EPR) effects (Figure 4) and facilitated endocytosis. In healthy tissues, the particles are unable to be transported through the narrow endothelial junctions of normal blood vessels; the defective tumor vasculature, however, show enhanced permeability [53]. The multiarm structure of the copolymers also allows the conjugation of specific ligands [52] to enhance permeability in biological membranes or therapeutic efficacy.

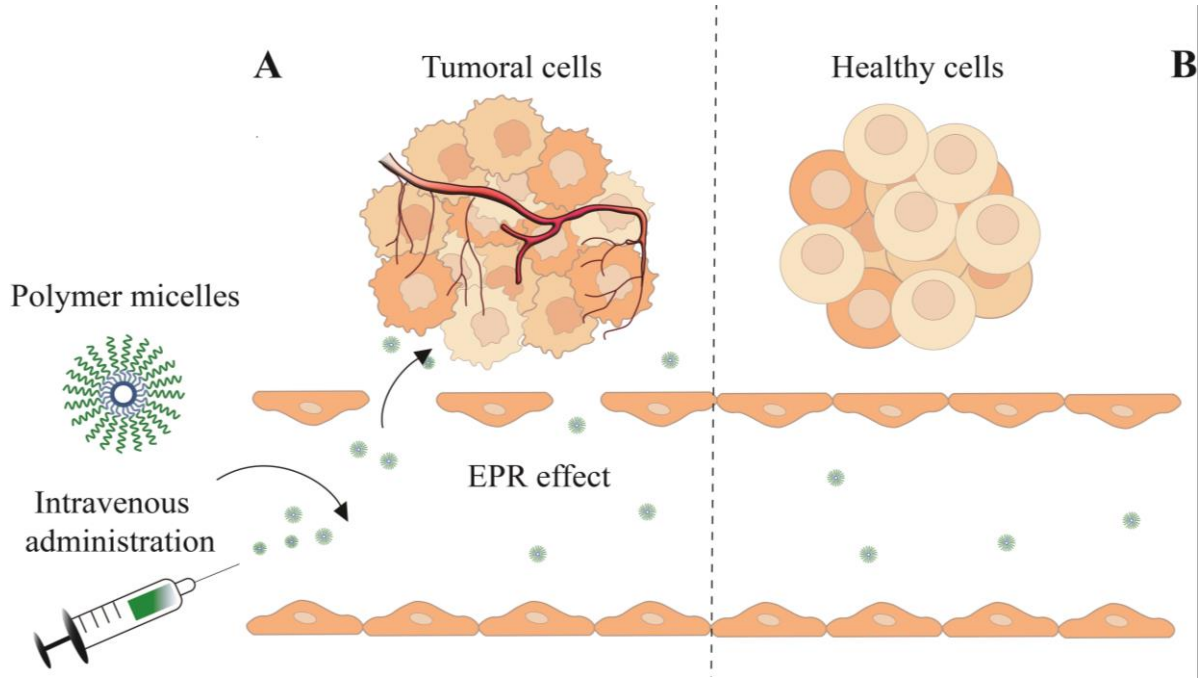

Fig. 4. Passive targeting of polymer micelles by enhanced permeability and retention (EPR) effect. Blood vessels in tumoral regions (A) are more permeable on particles diffusion than vessels close to healthy tissues (B).

Considering that micellar constructs of anticancer drugs as paclitaxel, cisplatin and epirubicin had already demonstrated high therapeutic efficacy and reduced adverse effects in previous clinical studies, Xian et al. (2015) applied this carrier for the delivery of the synthetic cannabinoid WIN55,212-2 (WIN). The main purpose of this study was to enhance the anticancer effect of this compound after nanoencapsulation. Breast and prostate cancer cell models were considered. Spontaneously associated styrene maleic acid-WIN micelles were prepared by mixing the pre-solubilized agents, followed by pH adjustments, filtration (to remove non-encapsulated WIN), freezing and lyophilization of the solution. The micelles had a ~15% loading, 132.7 nm average diameter, -0.0388 mV charge, and pH-dependent compound release profile (greater at pH 7.5 than 5.5). A slow-release rate of the cannabinoid was found at physiological pH, achieving only 30% after 96 h. This result suggest that systemic side effects would be avoided while a maximal amount of WIN would act in the tumor site. Pharmacokinetic studies are, however, needed to understand whether this amount of compound released is sufficient for the therapeutic effect. A dose-dependent inhibition of cell growth was observed in all three tumoral cell lines (MDA-MB-231, MCF-7 and PC3 cells) treated with both free and micellar WIN. The systems showed a similar cytotoxicity with at least 70% cell growth inhibition at 10 μM. Taken together, these *in vitro* results suggest that WIN micelles are promising nanocarriers in breast and prostate cancer [53].

In view of these positive results against breast cancer cells, WIN nanomicelles were also tested in a syngeneic mouse model of triple-negative breast cancer (TNBC). TNBC is a highly heterogeneous disease with no validated therapeutic target, and the response of the patients to the treatment is difficult to predict. Nanomicelles were prepared by the same method as described above, presenting similar characteristics (~18% loading, 152.1 nm average diameter and -0.08 mV charge). A slow-release rate of the cannabinoid was also found in water pH 7.4, achieving only 6.7% after 24 h. In fetal bovine serum, a higher release rate of WIN was found, i.e., 34.5% at 24 h. Both free and nanoencapsulated WIN induced the $p^{27/Kip1}$ protein expression, which lead to cell cycle arrest and apoptosis. A single injection in tail vein of nanomicelles increased the amount of WIN in the tumor tissues in approximately 5-fold compared to free WIN after 24 h. This finding may be explained by the EPR effect and the decreased metabolism. Mice treated for 12 days with 5 and 10 mg/kg of nanomicelles reduced the tumor growth by nearly 40% and 78%, respectively. Unlike the free WIN, WIN-loaded nanomicelles were not able to cross the blood-brain barrier, which results in lower occurrence of psychotropic adverse effects. These findings open new perspectives in the treatment of triple-negative breast cancer considering that low doses of nanomicelles could reduce both tumor growth and psychoactive effects - a critical factor for the anticancer applications of cannabinoid analogs [54].

Styrene maleic acid-WIN micelles have also been tested for neuropathic pain in rat model. The micelles were prepared by the method already reported, with a drug loading ranging from 5.7% to 27.0%. Micelles with higher EE showed a stronger hydrophobic association between the styrene moiety and WIN, leading to more stable constructs and slower release rate. Chronic constriction injury-induced mechanical allodynia was attenuated for up to 8 h at 11.5 mg/kg of WIN-loaded micelles, a prolonged period compared to non-encapsulated WIN. The rotarod assay was considered to evaluate central effect on motor function. Free and encapsulated form of WIN resulted in a similar initial impairment. The rapid onset of ataxia in rats after administration of micelles was unexpected, which might be associated with a rapid internalization of micelles in the brain or limitations in experimental protocols [55].

CBD has also been incorporated in polymer nanomicelles and used for cancer applications [56]. PEO_{101}-b-PPO_{56}-b-PEO_{101} (Poloxamer® 407) micelles were first prepared by solvent evaporation and then embedded in a cryogel carrier via UV-assisted cryotropic gelation of 2-hydroxyethyl cellulose. This polymer micelles presented a great average size of 173 nm. As the cryogel formulations were aimed for dermal application, *in vitro* release studies were performed at pH 5.5. An initial burst release was observed for the pure HEC cryogel carrier

(50% of CBD was released in 1 hour) and the release process was completed within 8 h. When cryogels with nanoparticles were tested, in turn, no burst effect was observed and the systems were characterized by a sustained release up to 24 h. No cytotoxicity was found for the proposed systems in mouse fibroblast cells. Nanocomposites cryogel carriers preserved the antineoplastic activity of CBD in assays with human tumor cell lines MJ (T-cell lymphocyte) and T-24 (urinary bladder carcinoma). Although only *in vitro* assays have been performed, the authors suggest that the nanocomposite cryogels are promising to treat skin lesions in cutaneous T-cell lymphoma and patients with recurrent non-invasive urinary bladder cancer [56].

3.2. Polymer nanoparticles

Polymer NPs (PNPs) are colloidal particles in the nanometer size that can be loaded with active compounds entrapped within or surface-adsorbed onto the polymeric core. They may be prepared from natural or synthetic polymers. Natural polymers provide greater biocompatibility whereas synthetic polymers show a greater control of drug release. In addition to a controlled drug release, polymer NPs may protect active molecules against degradation reactions (e.g., hydrolysis, oxidation) and improve both bioavailability and therapeutic index [57]. PNPs have extended the release of cannabinoids for many days or weeks, particularly in systems with surface functionalization [58,59] (Table 3). Consequently, an increased interest of these NPs in chronic pathologies such as cancer and CNS-affecting diseases has been observed in the last years.

Poly-lactic-*co*-glycolic acid (PLGA) NPs containing CBD were developed by the emulsion solvent evaporation technique for ovarian cancer applications. Given that ovarian cancer cells spread within peritoneal cavity, these particles were designed for an intraperitoneal administration. Cannabinoid receptors are overexpressed in epithelial ovarian cancer cells, suggesting the involvement of the endocannabinoid system in this pathology. In this context, cannabinoids as CBD have shown to be promising therapeutic alternatives. On the other hand, this compound is characterized by low chemical stability, and it is also rapidly depurated from the peritoneal cavity when administered as a solution. Nanotechnology has been purposed to overcome these limitations. Optimal CBD-NPs showed a spherical shape, a particle size of 236 nm, negative zeta potential value of -16.6 mV and high E.E. (> 95%). The authors suggest that this particle size would be enough to retain the systems in the peritoneal cavity and for cell internalization. In *in vitro* release assays, in turn, demonstrated an initial burst release at the

first hour, followed by a sustained release (achieved 100% of released active compound within 96 h). No changes in particle size, zeta potential and CBD content were found after storage for 3 months at 5 °C, suggesting that nanoencapsulation protects CBD from degradation. The internalization of CBD-NPs by SKOV-3 epithelial ovarian cancer cells occurred in a time-dependent manner, reaching a maximum after 4 h of incubation. CBD-NPs showed a lower IC_{50} values than the CBD solution, indicating improved antiproliferative effect after nanoencapsulation. The type of cell death induced by CBD was the apoptosis regardless of the sample tested. In an "ovo" model, CBD-NPs exhibited a slightly higher tumor growth inhibition compared to CBD in solution [59].

Poly-lactic-*co*-glycolic acid (PLGA) nanoparticles containing Δ^9-THC were developed by nanoprecipitation method and their surface functionalized with poly(ethylene glycol) (PEG), chitosan (CS) or a combination of these agents (PEG-CS) aiming to improve the intestinal absorption and minimize protein adsorption. Unmodified PLGA NPs presented a mean particle size between 259 and 434 nm, which increased after surface modifications (600-900 nm). The zeta potential was also affected by surface functionalization. For pure polymer NPs, this parameter was -30 mV, which changed to +0.5, +70 and +3 mV after surface functionalization with PEG, CS and PEG-SC, respectively. All formulations presented an E.E. value greater than 80%. FTIR analyses demonstrated that structure of the polymer was preserved after NP preparation and that Δ^9-THC was effectively incorporated into NPs. As these NPs were intended for oral delivery, *in vitro* release studies at pH 2.0 (gastric) and 7.4 (intestinal) were performed. Under acidic conditions, Δ^9-THC was released in a slow manner (<10% drug release after 2 h). All the PLGA-based NPs showed a biphasic profile in intestinal pH. The surface functionalization with PEG accelerated the Δ^9-THC release whereas the presence of CS on NP surface resulted in a opposite effect. The different physicochemical nature of these polymers justifies these results. Chitosan would create a hydrophobic barrier, limiting the water access and compound release. A negligible effect on the hemolysis rate (<2.7%) was found for all formulations. Moreover, the tested vehicles (blank formulations) do not appear to be cytotoxic in assays with Caco-2 cells. Surface functionalization positively impacted the rate of cell internalization. The internalization rate of PEG-CS-PLGA NPs and PEG-PLGA NPs in these cells was close to 27% and 12%, respectively. Although the presence of CS has reduced the Δ^9-THC release, its mucoadhesive properties positively impact on cell internalization rate [60].

This same research team incorporated Δ^9-THC into polymer NPs for cancer applications with intravenous administration. A surface modification with PEG, CS or PEG-CS was also

employed to minimize the opsonization phenomenon, and vitamin E was included into the formulations to reduce chemical oxidation. Spherical shape NPs were prepared by the nanoprecipitation method. The mean diameter of uncoated NPs, which was 290.1 nm, increased upon coating with PEG, CS, and PEG–CS up to 587.9, 746.89, and 789.67 nm, respectively. Uncoated NPs presented a zeta potential value of -34.78 mV. The surface electrical charge was controlled by the type of polymer coating onto the PLGA particles. Positive zeta potential values were obtained after surface functionalization, which were +0.46, +78.21 and +5.34 mV for PEG, CS, and PEG–CS NPs, respectively. E.E. values higher than 95% were achieved. A biphasic profile was found for the different systems. Unlike the PEG functionalization, CS prolonged Δ^9-THC release (e.g., about 10% vs. 60% of Δ^9-THC released in day 3) as already reported. Low hemolysis rate was observed for all developed systems. PEGylated PLGA NPs showed to be more cytotoxic against A-549 cells (adenocarcinomic human alveolar basal epithelial cell model) than PEGylated chitosan-coated PLGA NPs and PLGA NPs. Considering these results, the activity of THC-loaded PEGylated PLGA NPs was also tested in LL2 lung tumor-bearing immunocompetent C57BL/6 mice. This formulation reduced in 2.2-fold the tumor volume on day 32 compared the control (Δ^9-THC solution). As the PEGylation did not result in a significant improved antitumoral activity, the authors suggest new studies with ligands specific to receptors overexpressed onto the cancer cell membrane [61].

Another surface functionalization approach performed in a recent study was the coupling of transferrin (Tf) in Δ^9-THC-loaded PLGA nanoparticles. The high expression of Tf receptors in tumor cells mediates cellular uptake of iron, enhancing the tumor cell proliferation. After Tf coupling on particle surface, a reduction in zeta potential was observed as carboxylic acid groups of the NPs are involved in this chemical interaction. A similar E.E. was found between THC-PLGA NPs and Tf-THC-PLGA NPs (>90%), which suggest that surface functionalization do not affect the loaded Δ^9-THC amount. All the formulations presented a nanometer size (260–332 nm) and narrow size distributions (PDI <0.3). As previously reported, Δ^9-THC-PLGA-NPs and Tf-THC-PLGA showed a biphasic release profile. In the same way, blank NPs were not cytotoxic against Caco-2 cells. Tf-THC PLGA NPs were less cytotoxic than plain THC-PLGA NPs (17% vs. 88%). *In vitro* uptake studies in the presence of inhibitors and fluorescent microscopy studies suggested that both types of NPs were internalized through cholesterol-associated and clathrin-mediated mechanisms. Tf-THC PLGA NPs were more slowly internalized by cells than THC-PLGA-NPs, which would result in a more extended action at the target cell surface [62].

Naphthalen-1-yl-(4-pentyloxynaphthalen1-yl)methanone (CB13), a potent agonist for CB1/CB2 receptors, has also been incorporated into PLGA nanoparticles. This therapeutic agent can reverse neuropathic mechanical hyperalgesia, which has already been shown in rats. On the other hand, CB13 is characterized by low aqueous solubility (~0.001–0.002 mg/mL), a limitation that may be overcome with nanoencapsulation [58,63].

The first study with PLGA NPs of CB13 considered a complete formulation study [63]. The impact of polymer type (different molecular weights), surfactant concentration and initial cannabinoid amount on particle features was evaluated. The particle size of blank NPs ranged from 90 to 300 nm. The higher the hydrophobicity of the polymer and surfactant concentration, the smaller the particle size. Different amounts of CB13 (0,10% and 20% w/V) did not affect the particle size, which was kept close to 300 nm. The zeta potential values of blank NPs ranged from -24 to -44.8 mV. In presence of CB13, the zeta potential was slightly lower (-20 mV). High E.E. was also found (68-90%), particularly for NPs prepared with lower amounts of CB13. An inverse relationship between release rate and NP size was found. In contrast, low molecular weight and lactide content resulted in a less hydrophobic structure with increased rate of water absorption, hydrolysis, and erosion. No cytotoxicity was observed for unloaded and CB13-loaded NPs in human normal colonic CCD-18Co cell lines and human carcinoma T-84 cell lines after 24 and 48 h [63].

The negative surface charge of CB13-loaded PLGA NPs could prevent or reduce the interaction with the intestinal mucosa and thus a surface modification with different agents (chitosan, vitamin E, lecithin and Eudragit® RS) was used as an alternative to overcome such limitations. Surface-modified PLGA NPs were obtained through a nanoprecipitation method. A mean particle size distribution in the range 253-344 nm (compatible with an oral absorption) was found. The zeta potential values were strongly negative for uncoated PLGA NPs, slightly negative for lecithin- and vitamin E-PLGA NPs and strongly positive for chitosan and Eudragit-PLGA NPs. As these last two formulations presented improved mucoadhesion properties, a relationship between zeta potential and mucoadhesion was established. Interestingly, lower mucoadhesion of formulations was found in the upper part of the small intestine than in posterior segments (different intestine tissue samples were tested). E.E. values were higher than 70%, suggesting high association of CB13 in particles. All formulations showed a prolonged release of CB13 over 15 days without burst effect. Lecithin- and vitamin E-PLGA NPs provided higher release rate of CB13 than chitosan- and Eudragit®-PLGA NPs. In Caco-2 assays with these two last formulations, higher adsorption and cell internalization was observed for

chitosan-decorated NPs. Finally, biodistribution assays were performed in rats with these same optimal NPs. The authors observed that surface functionalization did not prevent the opsonization and particles showed a preferential distribution in the liver and spleen [58].

Table 3. Polymer nanoparticles with cannabinoids.

Polymer	Tested cannabinoid	Administration route	Particle size/ release profile	Main results in biological assays	Reference
PLGA	CBD	Intraperitoneal	236 nm >Burst initial followed by a sustained release (100% of release CBD within 96 h)	Nanoencapsulation of CBD increased antitumoral activity against ovarian cells (reduced IC_{50})	Fraguas-Sánchez et al. (2020)
PLGA For surface functionalization -chitosan and PEG	Δ^9-THC	Oral	294/434 and 600/900 nm before and after surface functionalization, respectively >Nanoparticles functionalized with chitosan had a lower release rate than those NPs pegylated. >Biphasic release profile in intestinal pH	NPs functionalized with chitosan presented higher cell internalization rate	Martin-Banderas et al. (2014)
PLGA For surface functionalization -chitosan and PEG	Δ^9-THC	Intravenous	≈290 and 587-789 nm before and after surface functionalization, respectively >Biphasic release profile >Chitosan functionalization slowed the release rate (10% of Δ^9-THC released in day 3)	Pegylated reduced the tumor volume in 2.2-fold on day 32 compared to Δ^9-THC solution (LL2 lung tumor-bearing immunocompetent C57BL/6 mice model)	Martin-Banderas et al. (2015)
PLGA For surface functionalization-transferrin	Δ^9-THC	Not mentioned	260-332 nm >Biphasic release profile (faster release in the first 10 h, followed by a slower phase achieving 50% within 140 h)	NPs functionalized with transferrin were more slowly internalized by cells.	Durán-Lobato et al. (2022)

PLGAs with different molar mass	CB13	Not mentioned	90-300 nm >Inverse relationship between release rate and NP size >Low molar mass and lactide content resulted in increased release rate	NPs were not cytotoxic against CCD-18Co and T-84 cells	Martín-Banderas et al. (2012)
PLGA For surface functionalization-chitosan and Eudragit®	CB13	Oral	253-344 nm >A sustained CB13 release was obtained for 15 days (no burst effect was observed)	Surface functionalization improved intestinal mucoadhesion.	Durán-Lobato et al. (2014)

Surface functionalization was performed for different purposes in studies presented here.

4. Inorganic nanoparticles

4.1 Metal nanoparticles

Metal NPs have gained attention due to their advanced features including optical properties, large surface energies, plasmon excitation, and quantum confinement. Natural resources have been often considered in the biosynthesis of green metal nanoparticles, which reduce toxicity problems. Plant extracts, for example, are composed of different compounds able to provide ideal capping, reducing, and stabilizing properties required for metal NP synthesis [64].

Cannabis sativa preparations has been considered for green synthesis of gold (AuNPs) and silver (AgNPs) nanoparticles, which are targeted to treat infections caused by biofilms. These complex biological structures secrete different surface molecules and virulence factors, which restrict the antibiotic diffusion and contribute to antibiotic resistant. In this context, AgNPs have demonstrated a promising role due to their high specificity of action (high activity and low toxicity). The reduced particle size allows to access the biofilm internal structure, increasing interactions with the bacteria. Metal NPs were synthesized from aqueous extracts of *C. sativa* stem separated into two different fractions (cortex and core [xylem part]). Cortex enriched in bast fibers was used in the synthesis of AuNPs, generating fiber-AuNPs (F-AuNPs). When the core part of the stem was considered, core-AuNPs (C-AuNPs) and core-AgNPs (C-AgNPs) were obtained. The later plant part is enriched with phenolic compounds such as alkaloids and cannabinoids, which play a role as reducing and stabilizing agents. The size of

nanoparticles ranged from 12 to 20 nm for F-AuNPs and C-AuNPs and between 20 and 40 nm for C-AgNPs. The zeta potential values of F-AuNPs, C-AuNPs and C-AgNPs were -12.3, -20.6 and -29.2 mV, respectively. NPs showed to be stable for up to 1 week storage. After that, the NPs diameter showed a minor reduction. The authors suggest that C-AgNPs presented strong antibacterial activity; however, no marketed drugs were included as control in the assays. MIC values of 6.25 and 12.5 µg/mL and MBC values of 12.5 and 25 µg/mL against *Pseudomonas aeruginosa* and *Escherichia coli* were obtained, respectively. On the other hand, this sample did not exhibit an inhibitory effect against *Staphylococcus epidermidis* at the tested concentrations. The different cell envelope composition may explain these findings. Gram negative bacteria such as *P. aeruginosa* and *E. coli* have a thinner cell wall and a double cell membrane, which facilitate the NP penetration. In biofilms, significant morphological changes were found after treatment with 100 and 200 µg/mL of AgNPs. The large surface area due to the reduced particle size of metal nanoparticles and the release of Ag^+ via oxidation (increases reactive oxidative species generation) may explain these findings of strong antibacterial activity [65]. AgNPs have also been obtained from leaf extracts of *Cannabis sativa*. In this new study, the authors found a synergistic action of these NPs with market antibiotics against *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumoniae* and *S. marcescens* [65].

A major drawback associated with plant extracts is the inadequate control and standardization of extracts, which may result in a non-reproducible NP synthesis. For this reason, CBD has also been tested as a reducing agent in metal NP synthesis. CBD-capped Ag and Au NPs showed to be spherical, monodispersed, and smaller than 10 nm. CBD-capped NPs presented lower cytotoxicity on HaCaT cells when compared to CBD alone. CBD-Au NPs resulted in higher cytotoxicity than CBD-Ag NPs (IC_{50} = 23.99 vs. >100 µg/mL). Although only *in vitro* assays have been performed, the authors suggest that CBD-Au NPs are promising in cancer treatment [66].

4.2 Carbon nanotubes

Carbon nanotubes (CNTs) are huge cylindrical large molecules presenting a hexagonal arrangement of sp^2 hybridized carbon atoms. The wall of CNTs is composed of a single (SWCNTs) or multiple layers of graphene sheets (MWCNTs). At the ends of the tubes, both SWCNTs and MWCNTs are capped in arrangement of carbon known as fullerenes [67]. Carbon nanotubes (CNTs) have emerged as the advanced nanocarriers for the delivery of various

compounds presenting short half-life or low aqueous solubility as cannabinoids [68,69]. In addition to improved solubility, CNTs may be functionalized with bioactive molecules, allowing a site-specific drug delivery and reduced toxicity [70].

MWCNTs, for example, have been purposed for 2-arachidonoyglycerol (2-AG) delivery. This cannabinoid has shown beneficial effects in colitis considering its action as a full agonist of CB1 and CB2 receptors, which are widely distributed in the gut. Once this compound presents a poor aqueous solubility as other cannabinoids and rapid hydrolysis, nanoencapsulation was considered to improve these aspects. In a rat model, colitis was induced by colonic instillation of trinitrobenzene sulfonic acid and the effect of MWCNTs on the colonic tissue damage and inflammation was evaluated. Unlike the free 2-AG solution, the intrarectal administration of MWCNTs-2-AG particles (2 mg/kg of 2-AG) 2 days before and 8 days after the induction of colitis reduced the macroscopic and microscopic injuries, malondialdehyde, TNF-α and IL-1β concentrations as well as myeloperoxidase activity (indicator of neutrophil infiltration into the inflamed and damaged tissue). The authors concluded that MWCNTs would protect 2-AG against the rapid hydrolysis, extending the therapeutic effect [68].

5. Comparative studies

Pharmacokinetic (PK) assays in humans testing different vehicles for CBD were also performed. CBD in a fixed dose (90 mg) was tested in a powder form (no dissolving vehicle), solubilized in sesame oil and after incorporation into self-nano-emulsifying drug delivery systems (particle size = 39 nm / PDI 0.3). In a powder form, an extremely low relative bioavailability, delayed T_{max} and variable PK profile were found. AUC values of CBD from SNEDDS and sesame oil were respectively 7 and 8-fold higher than the powder form. Sesame oil and self-nano-emulsifying systems presented a similar CBD plasma exposure. On the other hand, SNEDDS provided an earlier absorption of CBD than the sesame oil (T_{max}=2 vs. 4 h). Furthermore, SNEDDS showed to be able to provide a more uniform absorption profile than the sesame oil (floating oily droplets over the aqueous content of the stomach may be present) [71]. Although clinical trials did not show statistical differences in plasma exposure and C_{max} after treatment with SNEDDS and sesame oil containing CBD, sesame oil resulted in a higher AUC and C_{max} than SNEDDS in studies performed for the same research team in a rat model [72]. Oil administration in rats affects parameters of absorption and distribution. Taken

together, these results suggest that the oral absorption of lipophilic molecules such as CBD in a solubilized form is crucial for an enhanced absorption [71].

A comparative activity study between solid lipid nanoparticles (SLN) and NEs of Δ^9-tetrahydrocannabinol-valine-hemisuccinate (THC-VHS), a hydrophilic prodrug of Δ^9-THC, was also performed. In a normotensive rabbit model, the performance of these nanocarrier systems for glaucoma management was tested by considering a multiple-dosing protocol. The intraocular pressure (IOP)-lowering capacity of cannabinoids, particularly Δ^9-THC, have been investigated for decades. The most probable hypothesis suggests that Δ^9-THC provides a CB1 receptor-mediated vasodilator effect, contributing to the aqueous humor efflux. Once IOP-reducing and neuroprotective effects require an effective permeation of Δ^9-THC from outer layers of the eye to target tissues in the anterior and posterior compartments, different vehicles (nanocarriers) and structure modifications have been considered. THC-VHS-loaded SLNs composed of Compritol® 888 ATO, Pluronic® F-68, Tween® 80 and glycerin were prepared by the ultra-sonication technique. Tocrisolve®, which is marketed emulsion composed of a 1:4 ratio of soya oil/water and emulsified with Pluronic® F68, was used as vehicle to prepare THC-VHS-loaded NE. THC-VHS-loaded SLNs and THC-VHS-loaded NEs presented a particle size of 287.8 and 189.8 nm, respectively. Animals treated with SLNs had significantly lower IOP than untreated eyes, maintaining IOP below baseline until 360 min. THC-VHS-NE, in turn, produced a significant drop in IOP compared to the untreated eye only up to 90 minutes. Solid lipids could sustain the THC-VHS release, explaining the most lasting activity. Moreover, lipid-based colloidal carriers provide an occlusive effect and form a depot of active compound that extend the action. Interestingly, SLNs demonstrated greater effect on IOP in terms of both intensity and duration than marketed pilocarpine and timolol maleate ophthalmic solutions. In summary, the THC structure modification would facilitate the transport of the parent molecule through the ocular tissues and lipid-based nanocarrier would act as a long-acting depot [73].

6. Final considerations

Lipid and polymer-based systems represented the main colloidal carrier groups identified in this study. Lipid carriers have been shown to be more effective in improving the biopharmaceutical properties of cannabinoids whereas polymer NPs extend their time of action or provide a more sustained compound release.

Among the lipid carriers, the SNEDDS represented the most studied colloidal system. In addition to preventing drug precipitation phenomena in the GIT, these colloidal carriers enhance the oral absorption of cannabinoids though different mechanisms. The reduced particle size of these systems allows an effective transport through the enterocytes, reducing the time to achieve a maximum plasma concentration (T_{max}) with an increased peak plasma concentration (C_{max}).

The type of lipid considered in the colloidal suspensions also appears to impact on the transport mechanism of cannabinoids. The presence of long-chain triglycerides, for example, has contributed to improve the oral absorption through the lymphatic pathway. Metabolism inhibitors may also be included into these systems, extending even more the action of cannabinoids as already observed clinically.

For topical applications, lipid-based systems have been preferentially selected due to their high biocompatibility, particularly when biological lipids (e.g., phosphatidylcholine, phosphatidylserine) are considered for the composition of the formulation. For example, various studies have developed Δ^9-THC-loaded lipid carriers for the treatment of glaucoma, which have presented an intraocular pressure lowering activity close to or greater than market products.

Polymer NPs, in turn, have the advantage of providing a sustained drug release and greater stability for the encapsulated material. In situations as cancer and CNS diseases, a sustained and site-specific compound delivery is particularly desirable. The cannabinoid release rate can be adjusted depending on the type of polymer considering that they present different degradation rates. Poly(lactic-co-glycolic acid) was the copolymer more reported in polymer NPs with cannabinoids, which can provide a compound release for many days. A site-specific compound delivery, in turn, is achieved through different surface functionalization approaches. NPs coated with molecules able to selectively bind to receptors highly expressed in the blood-brain barrier or in tumor cells can make the release of cannabinoids more selective, reducing adverse effects on non-target tissues. Interestingly, the surface modification of NPs with CBD itself improve the interaction of these particles not only in tumor cells but also in the Central Nervous System. On the other hand, CBD is more susceptible to degradation via oxidation when it is not internalized.

Diseases affecting the blood-brain barrier integrity (most CNS diseases) and cancer also favor the application of NPs as drug carriers. In both situations, endothelial barriers are

compromised and then the transport of small particles is increased. Therefore, strict particle size control should be considered during the preparation of these systems.

Variable particle sizes and zeta potential were found for each type of nanoparticle with impacts in their applications. Micelles, for example, are characterized by a very small particle size, which could be interesting for cellular intercellular in cancer therapies. For the SNEEDS, the reduced particle size allows an effective transport through the enterocytes. When smaller nanoparticles are administered intravenously, they tend to accumulate in tumor tissues much more than in normal tissues due to enhanced permeability and retention (EPR) effect. The zeta potential, in turn, affect cell interaction or with other biological constituents. When nanoparticles are applied in mucosal regions (e.g., buccal or nasal), a zeta potential is desired to improve the mucoadhesion. In contrast, cationic nanoparticles have demonstrated to be more toxic than the anionic counterpart. The surface charge also affects the interaction level between NPs and plasma proteins or cells of the phagocytic system. Surface functionalization could be useful for controlling this aspect.

In summary, nanosystems are able to overcome several biopharmaceutical and pharmacokinetics limitations of cannabinoids (high lipophilicity, low bioavailability, high hepatic metabolism and non-specific distribution in adipose system). Besides that, nanotechnology may also provide a more sustained and targeted drug release, reduce toxicity and improve efficacy. Cannabinoid-based systems have shown promise for several difficult-to-treat diseases. Furthermore, the nanosystems could expand the routes of administration currently used for these therapeutic molecules. More translational studies are still needed to confirm all benefits found in *in vitro* assays reported here.

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