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SKIN PERMEATION STUDIES USING NEW NANOFORMULATIONS CONTAINING CANNABIDIOL FOR THERAPEUTIC PURPOSES

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Abstract: A possible route of cannabinoid administration is the topical-transdermal route. In this research, a chromatographic method to identify cannabidiol was developed, studies were also carried out using Franz cells and Confocal Raman Spectroscopy with two unprecedented nanoformulations (TRANS and CLN TRANS) containing cannabidiol (CBD). The CLN TRANS formulation presented a greater amount of CBD in the stratum corneum (SC) of the skin than TRANS. However, in the epidermis and dermis the result was the opposite. The peak intensities in Raman spectroscopy showed an equal amount of CBD in the dermis of the skin with both formulations until 4 hours of application, but the intensity of CBD decreased on the skin where CLN TRANS was applied after 8 hours. Therefore, the TRANS formulation continued to penetrate the human skin to deliver CBD deeper than CLN TRANS, showing promising results as a candidate for pain relief in patients.
Keywords: Nanomedicine; Cannabinoids; Dermatology; Enhanced skin delivery; Raman spectroscopy; Topical drug delivery.

INTRODUCTION

The use of plant-derived extracts to relieve pain like *Cannabis sativa* has been used medicinally for a long time [1]. Cannabis contains a combination of cannabinoids, including about 125 substances called phytocannabinoids, among them the major constituents are delta-9-tetrahydrocannabinol (D9-THC). It exhibits psychotropic effects, and the other cannabinoid that has been attracting attention is cannabidiol (CBD), which presents itself as a therapeutic agent with analgesic, anxiolytic, anti-inflammatory properties, among others [2-5].

Cannabidiol (CBD), an important non-intoxicating phytocannabinoid, seems to be effective in controlling pain, especially

in those considered as pain mediated by the endocannabinoid system, and it is the main cannabinoid associated with therapeutic use without generating any psychoactive effect [2-4,6]. Because of its medicinal effects, cannabis for medical use has been authorized for research and therapeutic use by several countries around the world [7]. Moreover opioids, currently used for chronic pain can lead to physical dependence, addiction and death by overdose. Because of these problems, interest in the properties of *Cannabis sativa* as a therapeutic alternative has increased, as well as legalization in several countries has facilitated its study [8].

The Endocannabinoid system focuses mainly on two cannabinoid receptors known as CB1 and CB2. The first one is responsible for most of the psychotropic effects, the CB1 receptor is distributed across various regions of the brain, most abundant in the Central Nervous System (CNS). CB2 receptors have their majority expression in the immune system, in microglia and in pathological conditions such as chronic pain [9-12].

CB1 and CB2 receptors can be activated directly or indirectly, and studies point to their relationship with pain. Activation of CB1 and CB2 receptors is believed to have antinociceptive effects and that inflammatory conditions of chronic pain are controlled by receptors relieving pain symptoms. As the CB2 receptor is less present in the brain, selective agonists and antagonists for this receptor have been studied and synthesized. Aiming to have minor psychological effects with greater effects to decrease pain (analgesic activity) [11, 13]. CB1 and CB2 receptors are also found in the skin and the fact that these receptors are related to pain, this demonstrates the potential of cannabidiol (CBD) for use in relieving pain and local inflammatory processes [7,14].

Transdermal administration of cannabidiol is not currently a clinical practice, but it is a potential alternative, especially in the context of adverse effects. An *in vitro* study carried out with human skin showed a permeability of CBD 10 times higher than that of THC. In this sense, patients will be able to absorb better the effects of CBD without the occurrence of the negative effects of THC [15]. The use of cannabinoids for the treatment of chronic pain is not recent, but evidence regarding efficacy, safety and mechanisms of action are still scarce [16]. In this sense, further research is needed in different routes of administration, such as the transdermal route, in order to increase the bioavailability of CBD and improve the effects of CBD in chronic pain patients.

Transdermal systems allow the delivery of drugs through the skin to the systemic circulation, circumventing the limitations of conventional administration routes, in addition to increasing therapeutic efficacy. The oral route has lower bioavailability. The intravenous route is a very invasive and may require hospitalization, which reduces therapeutic adherence. The transdermal route can contribute to the reduction of negative effects related to the pharmacokinetics and pharmacodynamics of drugs, for example by preventing the drug from being broken down by intestinal enzymes and first-pass hepatic metabolism. In addition to maintaining the plasmatic level of the drug for a longer period and providing the controlled and prolonged release of the active principles [17,18].

The cannabidiol (CBD) can be administered by different routes, however, due to their lipophilicity, it is necessary a vehicle that allows CBD administration in aqueous solution (for example in topical use to permeate the skin). Thus, nanotechnology has been used to develop nanocarriers capable of encapsulating and conducting the drug to therapeutic targets. In addition to being able to increase hydrophilicity and physicochemical stability, facilitating skin permeation [19,20].

One of the possible routes of administration for cannabinoids is the topical-transdermal route, which avoids first-pass hepatic and/or intestinal metabolism. Additionally, this route allows the drug to be delivered for a longer period and minimizes its concentration peaks that cause adverse effects, being potentially ideal for treating local symptoms such as pain and inflammation [21-23]. To achieve the goal of treating these symptoms, some strategies in the development of formulations using nanocarriers are commonly studied with the intention of making the drug less susceptible to degradation (for example, by the action of light, temperature, and oxidation) and also more soluble in water. Examples of strategies are micellization, nano/micro-emulsification and encapsulation in lipid-based formulations (liposomes, transferosomes and nanostructured lipid carriers) [9, 24,25].

Nanostructured lipid carriers (NLCs) are a second generation of lipid nanoparticles. Nanostructured lipid carrier particles are produced using mixtures of solid lipids with liquid lipids (oils), preferably in a ratio of 70:30 to a ratio of 99.9:0.1. Its main advantage over solid lipid nanoparticles (SLNs) is the minimization of the potential expulsion of active compounds during storage [26]. They have the characteristics of being nanoparticles composed of physiological and biodegradable lipids, with low toxicity and cytotoxicity, which means excellent tolerability, in addition to moisturizing the skin [26]. They are also deformable nanostructures capable of protecting unstable and sensitive drugs, have high physicochemical stability, and can reduce the amount of drug needed to produce the therapeutic effect by increasing the bioavailability of the encapsulated drug [27,28].

Due to their characteristics, nanostructured lipid carriers (NLCs) are a promising strategy option for cannabidiol encapsulation, making it more stable and capable of acting topically, which has been showing promising results. As demonstrated by Esposito et al. who formulated NLC with cannabidiol, the formulation was able to maintain the morphology of the nanoparticle similar to the non-encapsulated one and presented encapsulation efficiency above 90%, therefore being suitable for biological studies [29].

Transfersomes are a new generation of liposomes, they are mainly formed by phospholipids, have greater encapsulation efficiency, are more flexible (up to 8 times more malleable compared to common liposomes) and due to their amphiphilic nature, these particles can incorporate hydrophilic drugs in their inner layer and lipophilic drugs in their lipid bilayer. In addition, transfersomes are very versatile, being able to be modified in size, surface, lipid composition and aqueous composition, therefore presenting potential to overcome the stratum corneum and hypodermis layer (lipid pathway) of the skin, thus increasing the delivery of therapeutic agents to treat diseases [30-32].

Transfersomes are deformable and flexible due to surfactants in their structure. Surfactants are responsible for modifying, at least, three properties of transfersome particles: elasticity, encapsulation efficiency and particle size. The choice of surfactant will depend on the drug to be encapsulated [30]. Edge activators are often single-chain surfactants that destabilize the lipid bilayers of vesicles and provide a flexible membrane, eventually making the transfersomes highly flexible, which maintains the integrity of the vesicle when it penetrates the cell layers of the skin, increasing the permeation power [33-35].

Most transfersomes (elastic liposomes) contain phosphatidylcholine, it is the most abundant lipid component of the cell membrane and is therefore highly tolerated by the skin, which decreases the risk of

undesirable effects such as hypersensitivity reactions. Due to their characteristics, transfersomes have been studied as drug carriers for different types of compounds, both hydrophilic and lipophilic [31,36].

The application of transfersomes does not involve any intricate procedures and they can be applied by a non-occluded process, whereby they pass through the entire multilayered lipid matrix of the stratum corneum because of hydration or osmotic force within the skin [37,38]. In addition, transfersomes can create drug depots in the skin for sustained drug release, delivering therapeutic agents into deeper layers of the skin or transporting drugs into the systemic circulation, depending on the concentration of the drug in the vesicles [39]. Therefore, transfersomes have good potential to provide an entirely new perspective for rational drug administration [40].

Drugs are applied to the skin to act locally (epithelial surface), dermatologically (skin tissue) or transdermally (systemically in the body), and the medicine can penetrate the skin directly through the stratum corneum (outermost part of the skin formed by corneocytes) or through follicles hair, sebaceous glands and sweat glands [27]. Aiming to improve drug delivery and overcome the skin's poorly permeable barrier, very small particles, such as nanoparticles made with lipids (NLCs) and transfersomes are strategies for creating new formulations with the potential to cross the skin. An example is the use of Franz cells to simulate the retention and permeation of the drug in the skin (*in vitro*), a study with cannabidiol nano emulsion showed a small amount of CBD in the receptor medium and no toxicity in keratinocyte cells [19].

Despite its benefits, the Franz diffusion cell has some disadvantages that should be considered in permeation studies. Among them, the Franz cell does not consider the *in vivo* metabolism of the skin, which influences the biotransformation and elimination of compounds, and it does not provide the permeation profile through different skin layers. In this context, an approach that considers these parameters is of utmost importance for cutaneous permeation analysis.

Among the modern techniques, Confocal Raman Spectroscopy (CRS) has emerged as an analytical method for the analysis of materials, including biological tissues [41]. The use of CRS for *in vivo* human skin analysis was pioneered by Caspers et al. [42], and is now accepted as a "gold standard" technique for the *in vivo* study of the human skin. CRS is based on the inelastic scattering of the light, rather than on its absorption. During a Raman analysis, the studied matter is illuminated by a monochromatic laser light, stimulating interaction between the incident photons and the molecules of the sample, resulting in light scattering. In the analysis of biological tissues such as skin, the incident light excites the molecules of the sample, and the resulting signal is captured to form a spectrum, which represents a biochemical map of the human skin components. Furthermore, confocal raman spectroscopy is a non-invasive method that can be used *in vivo*, providing high spatial resolution in microns (μm) and the ability to determine the permeation profile of formulations in different skin layers.

Therefore, the objective of this research work was to develop two new formulations (NLC and transfersome), on a nanometric scale, containing cannabidiol, and combine both in synergy, one part of NLC to one part of transfersome (1:1) to obtain a product with greater capacity to continue releasing the drug into the skin, and with better permeation propensity to act as an analgesic (topical treatment of chronic pain). Thus, in addition to the new formulations developed, studies *in vitro* using Franz cell, and *in vivo* using

Confocal Raman Spectroscopy were carried out. We do not present many details about the formulations developed, as they are industrial secrets.

MATERIALS AND METHODS

IDENTIFICATION OF CANNABIDIOL

a) Chromatographic Conditions

Waters™ ACQUITY UPLC H-Class PLUS System. Mobile phase: ethanol/water (88:12); Chromatographic Column: C18; Column temperature: 50°C; Mobile phase flow rate: 0.42 mL/minute; Injection volume: 3µL; Total analytical run time: 3 minutes; Hold time: 1.1-1.2 minutes; Electrospray mode with negative ionization; Molecular weight of analysis: 313 (M-H). Method developed based on a previous study carried out in high-performance liquid chromatography [43,44].

b) Development and Validation of the Analytical Method

A fast and sensitive Ultra Efficiency Liquid Chromatography coupled to Mass Spectrometry (UPLC-MS) analytical method was developed and validated for detection of CBD in skin permeation and retention samples after *in vitro* permeation assays on pig ear skin. In the mass detector, the negative mode was chosen looking for the molecular weight of 313(M-H) considering that the molecular weight of CBD is 314.

c) Linearity

A stock solution of CBD diluted in methanol was prepared at a ratio of 1 mg of CBD to 10 mL, generating a concentration of 100 µg/mL of CBD. Then, this solution was diluted 100x (10 µl of the stock solution + 990 µl of methanol), thus obtaining a final concentration of 1 µg/mL equivalent to 1000 ng/mL. To prepare the analytical curve, the 1 µg/mL solution was used to carry out several dilutions in the concentration range of 500 to 10 ng/mL. The CBD analytical curve was plotted as a function of CBD mass per concentration in ng/mL. The linearity of the method was confirmed considering a linear correlation coefficient greater than 0.98.

d) Limit of detection and quantification

The limit of detection and quantification of the analytical method was evaluated from several dilutions at a concentration of 50 ng/mL of CBD in methanol, obtaining 25, 10 and 5 ng/mL. For quantification, the lowest concentration of CBD analyzed with linearity and precision was selected, while for the limit of detection the criterion used was visual, with the signal being 2 to 3 times the noise size of the baseline.

e) Precision and Accuracy

The precision and accuracy of the analytical method was performed by analyzing 3 different concentrations of the analytical curve, namely the concentrations of 50, 200 and 500 ng/mL (n=3) analyzed on the same day and on three different days (n =9). Precision results were expressed by coefficient of variation (CV%) admitting values less than or equal to 10% according to the formula:

CV% = (standard deviation/mean concentration determined) X 100

The accuracy results were expressed by the ratio between the average concentration determined experimentally and the corresponding theoretical concentration following the formula above and should be between 80 and 120%.

f) Selectivity

To evaluate the selectivity of the analytical method, the samples that could result in possible interferences in the CBD analyzes were evaluated. The interferers studied were extraction solvent of CBD (methanol), the mobile phase, methanol plus adhesive tapes containing stratum corneum (white stripping tape), methanol plus Epidermis+Dermis skin homogenate (white retention), and the PBS solution (receptor medium).

ELABORATED AND TESTED FORMULATIONS

a) Nomenclature

The investigated products were transferosome containing isolated cannabidiol and lipid-based nanocarrier associated to the transferosome containing isolated cannabidiol in a 1:1 ratio (Table 1). The three formulations were optimized and presented the best results in terms of particle size, uniformity and stability.

Formulation	Assigned nomenclature
Transfersome containing isolated cannabidiol	TRANS
Lipid-based nanocarrier (NLC) containing isolated cannabidiol	CLN
Lipid-based nanocarrier (NLC) associated to a transfersome containing isolated cannabidiol (1:1)	CLN TRANS

Table 1. Investigated formulations.

b) Production of nanoparticles containing CBD and particle size

The lipid nanoparticle formulation (NLC) used 1% cannabidiol for encapsulation (named as CLN). The transfersome contains 2% cannabidiol (named as TRANS). The formulation named as CLN TRANS was developed by mixing, 10 grams (g) of the CLN formulation with 10g of TRANS.

The average particle size and polydispersity index of the three formulations were analyzed by DLS measurements using the Malvern Zetasizer Nano-ZS device. The formulations were diluted in water for the test.

IN VITRO PERMEATION

Permeation studies were carried out using pig ear skin, as animal biological membrane, obtained from the FRIGODELISS slaughterhouse. For the *in vitro* assays, the skin was dissected on the same day the animal was slaughtered, and kept frozen at -20°C.

Automated modified Franz-type Vertical Diffusion Cell (VDC) equipment (Phoenix – Research Corporation®) was used for the *in vitro* assays. Sodium phosphate buffer solution with pH 7.4 and 10 % ethanol was used as receptor medium per diffusion cell (14 mL), which was maintained at 32°C and constant agitation (400 rpm) throughout the experiment.

The skins were dermatomized to a thickness of 500 µm using a dermatometer. To ensure standardization in the thickness uniformity of all skin membranes used in the tests, a caliper was used. Previously dermatomed skins were evaluated for their thickness. Then, the electrical resistance of the skins was evaluated using EVOM2 Epithelial voltohmmeter equipment. For this, pieces of skin were applied to the inserts covering the entire base of the insert, and then saline sodium phosphate buffer solution with pH 7.4 was applied below and above the tissue, avoiding contact between the solutions. The electrodes were fixed in the compartments below and above the skin and the resistance was measured in the equipment.

The skins with suitable thicknesses, triplicate (n=3), were placed in a Franz cell and 0.5g of formulation was applied on the stratum corneum. The formulations used in the study were the transfersome (named as TRANS), and the mixture (1:1) of transfersome with nanostructured lipid carrier (named as CLN TRANS). After 3, 6, 9 and 12 hours of permeation, automatic collections were performed from the permeate, and the solution was analyzed by UPLC-MS to quantify the CBD present. The same volume of permeate solution that was withdrawn at each collection time was automatically replaced with freshly prepared PBS solution. Thus, keeping the volume of the Franz cell constant throughout the experiment.

The results were analyzed based on the analytical curve. Analyzes were presented as CBD concentration, permeated through the skin, and per permeation area (ng/cm^2), at each collection time. Therefore, demonstrating a CBD permeation profile.

After 12 hours of the *in vitro* skin permeation test, the skins were removed from the Franz type vertical diffusion equipment. Paper was used to remove excess formulation from the skin for retention studies. Then, the tape stripping procedure to remove the stratum corneum (SC) layers was performed [45,46] using 10 adhesive tapes (3M, Scotch®), which were transferred to test tubes containing 5 mL of extraction solvent (methanol). The remaining Epidermis+Dermis tissue (without SC) was cut into small pieces and transferred to tubes containing 15 mL of methanol. The skin samples were crushed in a homogenizing equipment (TURRAX®) for 1 minute to obtain a skin homogenate, then sonicated for 15 minutes in an ultrasound bath and filtered through a 25 mm PVDF syringe filter, with pores of 0.22 µm. The final solution was analyzed in UPLC-MS to quantify the CBD present in both SC and Epidermis+Dermis layers. The results were analyzed based on the analytical curve and presented as the concentration of CBD retained in the skin (ng/cm^2).

RAMAN SPECTROSCOPY AND DATA ANALYSIS

This research has been approved by the ethics committee of Universidade Brasil (CAAE: 67375323.6.0000.5494, number 5.921.917). Single-center, comparative, randomized, double-blind, instrumental clinical study aimed at investigating the skin permeation of nano-encapsulated cannabidiol through human skin by Confocal Raman Spectroscopy. The study is controlled and requires specific temperatures and air humidity, all the rooms to be used are prepared for the assessments. The experiment was carried out at DermoProbes – Skin, Hair and Nail Technology, São José dos Campos, SP, Brazil.

Ten (10) research participants were selected from DermoProbes database based on the inclusion and non-inclusion criteria. *Inclusion criteria*: Gender independent; Aged between 45 to 55 years old; Intact skin at the region to be analyzed; Be phototype II or III according to Fitzpatrick classification; Agreement to adhere the clinical procedures and to be present at the research center at the determined days and times for the measurements; Staying the seven days before measurements without applying any product at the regions to be analyzed; Signature of the Free and Informed Consent Form (IC) approved by the Research Ethics Committee. *Exclusion criteria*: Pregnancy/ lactation or intention to become pregnant during the study period; Use of some topical or systemic medications (immunosuppressants, antihistamines, nonsteroidal anti-inflammatory drugs, and corticosteroids within 30 days prior to the measurements, or immunosuppressants within 3 months prior to the measurements; History of atopic or allergic reactions to cosmetic products; Skin cancer cases of the participant or inn the participant's family members; Active skin pathologies and/or lesions (localized and/or widespread) in the evaluation area; Sensitivity or irritation to sunlight; Use of cosmetic products in the evaluation area within 7 days prior to the measurements; Marks in the experimental area that may interfere with the evaluation; Known history or suspected intolerance to products of the same category; Aesthetic or dermatological treatment in the evaluation area within two weeks prior to screening.

A Raman system gen2-SCA from *RiverD International* B.V. was used in the study. Laser light was centered at the skin surface within the delimited region to be analyzed with an

objective of 40x, located under the quartz window. Wavelength was set at 785nm. Raman signal was collected by a Charge Coupled Device (CCD). Laser power was set at 20 ± 2 mW, considered safe, not causing any side-effect. Spectral acquisition occurred within the low frequency region, also known as the fingerprint region, between 400 and 1800 cm-1. Analyzed depths ranged between 0 to 120 µm, covering stratum corneum, epidermis and dermis. At the stratum corneum, depth range was between 0 to 20 ± 2 µm with steps every 2 µm; at the epidermis (EP), depth range was between 20 to 70 ± 4 µm with steps every 4 µm; at the dermis, an isolated depth was chosen for spectral collection were a single point around 120 ± 5 µm was chosen. Before each analysis, climate conditioning and preparation of the measurement area were carried out. The research participant spent 30 minutes in a climate-controlled environment (23°C ± 1°C) with relative humidity maintained between 40-50%. The measurement areas, a 1 cm^2 region on the right and left forearms, were delineated prior to the start of the measurements at T0, using micropores and it has been cleaned with optical paper.

Measurements were taken at three distinct time points: T0 (baseline - no product applied), T4 (4 hours after product application), and T8 (8 hours after product application), resulting in a total of four (4) visits to the research center for each participant.

The Raman data obtained in the low-frequency (fingerprint) region were preprocessed using an algorithm by Python Script. A Savitzky-Golay smoothing filter, size 9 and degree 5, was applied. Fluorescence was eliminated by baseline subtraction using a polynomial fit of degree 7.

After background subtraction, all Raman data was vector normalized. For SC and EP, specific band intensity changes were identified at 1296 and 1440 cm^{-1}, and the intensity was determined for each time (T0, T4 and T8) and plotted versus the skin depth. At the dermis, once an increase in the Amide I peak intensity was identified, the integrated area of the average of the Amide I band region (1510 – 1720 cm^{-1}) was calculated for T0, T4 and T8. Later, T4 and T8 were subtracted from T0. This allowed the observation of the net effect of TRANS and CLN TRANS increase at T4 and T8 compared to baseline T0. A multivariate statistical analysis, Principal Component Analysis (PCA) was performed. In this analysis, T0 was initially compared to T4, and later with T8. Loading plots were then generated to identify the spectral regions of interest that were related to the separation observed between groups at the PCA.

RESULTS

The formulations (CLN, TRANS and CLN TRANS) were produced to have a gelatinous consistency for better application to the skin.

AVERAGE PARTICLE SIZE

The CLN formulation had an average particle size of 189 nm and a polydispersity index (PDI) of 0.16 (Figure 1).

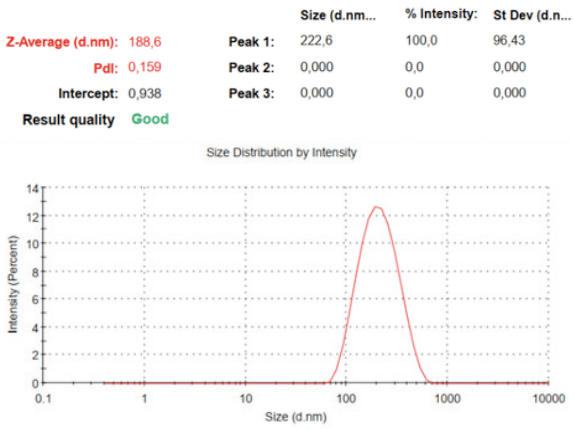

Figure 1: Average particle size of CLN containing CBD

The TRANS formulation had an average particle size of 162 nm and a polydispersity index (PDI) of 0.23 (Figure 2).

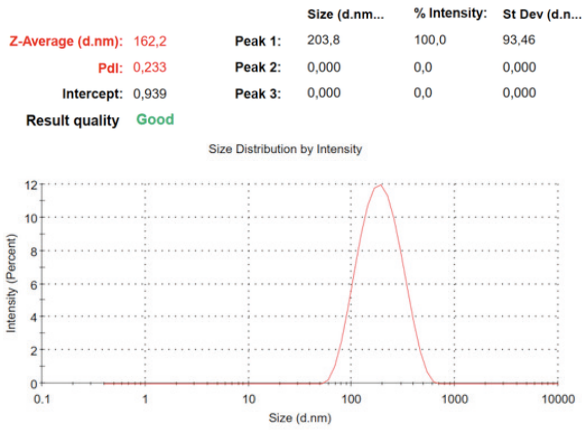

Figure 2: Average particle size of TRANS containing CBD

The CLN TRANS formulation had an average particle size of 234 nm and a polydispersity index (PDI) of 0.40 (Figure 3).

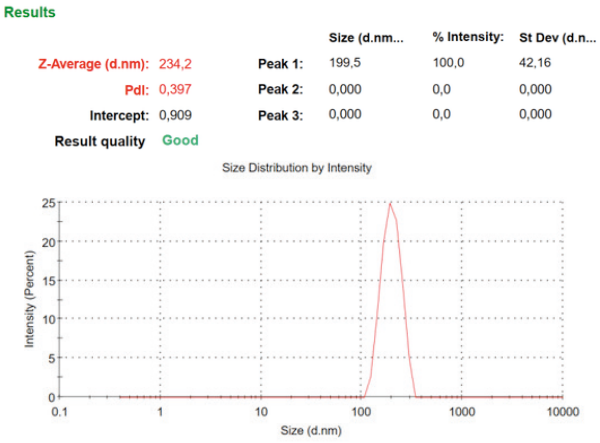

Figure 3: Average particle size of CLN TRANS containing CBD

IDENTIFICATION OF CANNABIDIOL

Mass-coupled liquid chromatography (UPLC-MS) analysis identified cannabidiol, molar mass 314 g/mol, at retention time of 1.1-1.2 minutes (Figure 4a). Moreover, the analytical curve of cannabidiol was obtained with linearity (Figure 4b).

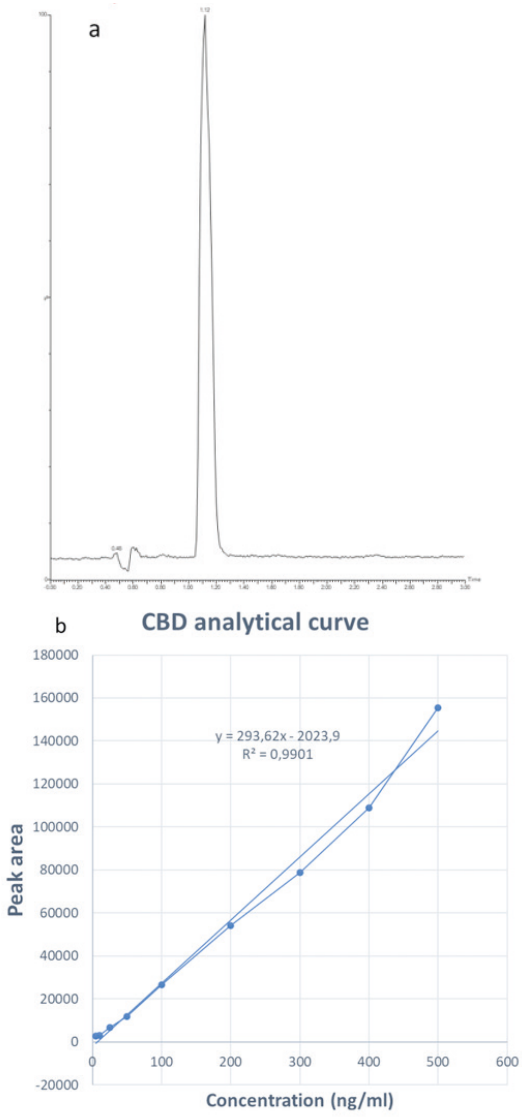

Figure 4: a) Cannabidiol Identification; b) Analytical curve in UPLC-MS

IN VITRO PERMEATION

The experiments carried out in triplicate showed that transfersome (TRANS) was able to deliver the highest permeation (transdermal release) of cannabidiol to the skin (Figure 5) after 12h (582 ng/cm^2). As expected, CLN showed the lowest permeation into the skin (176 ng/cm^2). While the NLC with transfersome (CLN TRANS) demonstrated slightly lower amounts of CBD permeation compared to the transfersome alone (342 ng/cm^2).

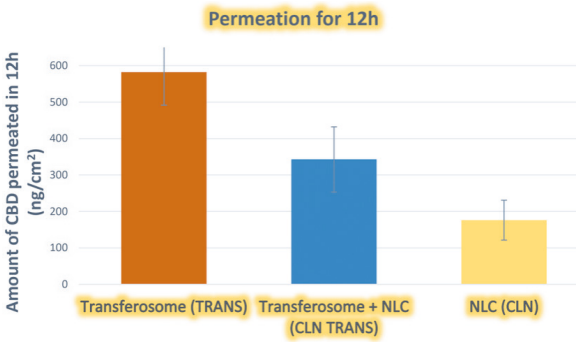

Figure 5: Cannabidiol permeation after 12h in Franz cells.

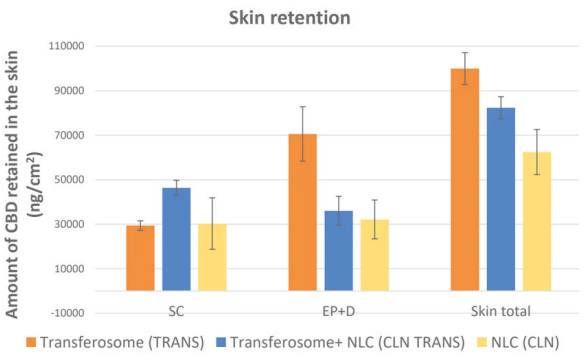

Figure 6: Cannabidiol retained in skin after 12h in Franz cells.

Cutaneous retention studies, with pig ear skins, performed after permeation showed that the amount of cannabidiol in the stratum corneum (SC) was more present in tests with CLN TRANS (46.330 ng/cm^2). While the TRANS formulation (29.394 ng/cm^2) and the CLN formulation (30.288 ng/cm^2) did not present statistically significant differences (Figure 6). In this experiment, the 10 adhesive strips used to remove and to quantify CBD in the stratum corneum were sufficient. Because, applying more tapes would tear the skin.

On the other hand, in the epidermis and dermis (EP+D), the transfersome (TRANS) delivered the highest amount of CBD (TRANS = 70.551 ng/cm^2). As CLN (32.152 ng/cm^2) and CLN TRANS (36.024 ng/cm^2) did not show significant differences in retention (Figure 6).

Thus, the difference in the amount of CBD retained in the skin is noticed when the SC and EP+D (Total Skin) results are added together (Figure 6), with a slightly higher prevalence of CBD in the skin with the TRANS formulation (TRANS= 99.946 ng/cm^2) compared to CLN TRANS formulation (CLN TRANS= 82.354 ng/cm^2). CLN showing the lowest CBD retention in pig ear skin (62.441 ng/cm^2).

RAMAN SPECTROSCOPY AND RAMAN DATA ANALYSIS

TRANS, CLN TRANS and CBD spectral signatures are displayed at figure 7, respectively. They were posteriorly used in the identification of the products permeation through the human skin.

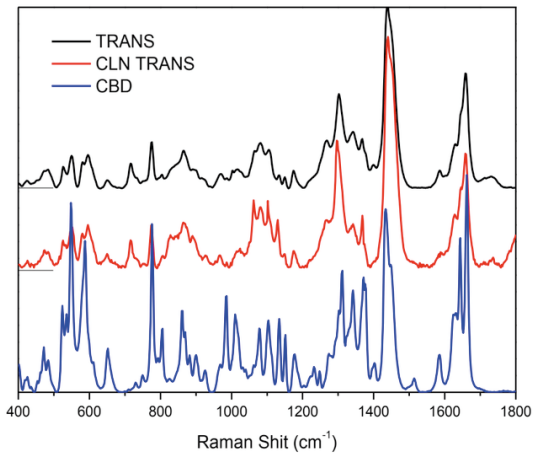

Figure 7: TRANS, CLN TRANS and CBD spectral signatures

For the TRANS formulation, it was analyzed the 1440 cm^{-1} peak intensity from stratum corneum to epidermis. As the CLN TRANS is a combination of TRANS and NLC (1:1), it was necessary a different approach in the permeation quantity for this case. The first step was to calculate the permeation profile for peak 1296 cm^{-1}, representing the NLC marker, and to calculate the permeation profile for

the peak 1440 cm^{-1}, representing the TRANS marker of this formulation.

To compare the two formulations (TRANS and CLN TRANS), it was performed an analysis by skin layer as shown on figures 8 and 9 for stratum corneum and epidermis, respectively.

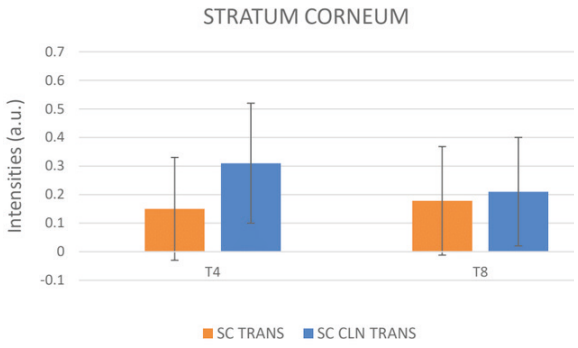

Figure 8: Comparison between TRANS and CLN TRANS at stratum corneum (SC). Intensity for peak 1296 cm^{-1}, representing the NLC marker, and intensity for the peak 1440 cm^{-1}, representing the transfersome (TRANS) marker.

By comparing the TRANS and CLN TRANS on SC, it can be seemed that the amount of CLN TRANS is 51,5% and 15% larger than TRANS at T4 and T8, respectively. However, the results presented a large standard deviation (dispersion), thus, they were not results with statistical significance (Figure 8).

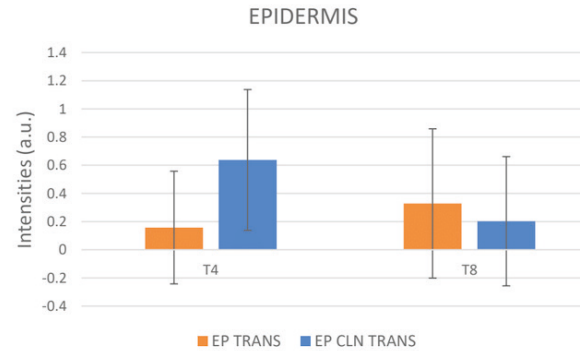

Figure 9: Comparison between TRANS and CLN TRANS at epidermis (EP).

At the epidermis the percentage of CLN TRANS at T4, is about 75% greater than TRANS. However, at T8 is observed a different trend for CLN TRANS. At this case, the quantity of CLN TRANS is about 62% smaller than TRANS. This probably indicates that the TRANS part of CLN TRANS continued to penetrate and, the NLC part, remains at the epidermis. The TRANS behavior indicates that it is still penetrating the skin to deliver CBD at the dermis. Again, the results in the epidermis presented a large standard deviation (dispersion), thus, they were not results with statistical significance (Figure 9).

At the dermis, it was observed a difference in the Amide I peak intensity (1662 cm^{-1}) for both TRANS and CLN TRANS formulations at the studied times. The integrated area of the band, considering the region between 1510 cm^{-1} and 1720 cm^{-1} was performed for both TRANS and CLN TRANS at times T0, T4 and T8. Later, a subtraction of times T4-T0 and T8-T0 was calculated (Net), and normality test for the subtracted data was performed, showing normal distribution for all. It was observed the presence of CBD in the dermis of both formulations, at both times, in relation to basal time T0. However, the CLN TRANS formulation showed an accentuated decrease, in the presence of CBD in the dermis, between T4 and T8 (Figure 10).

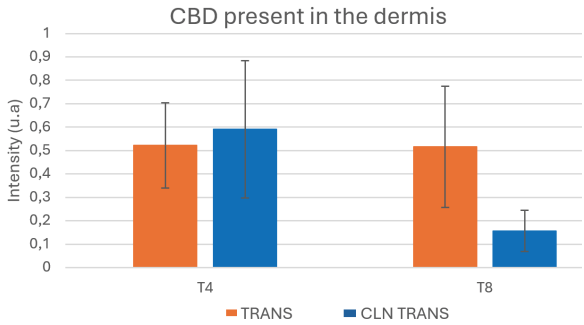

Figure 10: CBD presence in the dermis calculated by the integrated area subtraction (T4-T0 and T8-T0) for TRANS and CLN TRANS.

DISCUSSION

A study conducted by Akram and collaborators carried out a literature search reporting several therapeutic applications of transferosomes. For example, transferosomes were efficient as carriers of anti-cancer agents, as carriers of agents with antifungal activity, and as carriers of agents with analgesic and anti-inflammatory activity [30]. For the treatment of rheumatoid arthritis, two studies, using transferosomes as carriers, showed excellent anti-inflammatory activities through deeper penetration of the gels into the skin layers [47,48]. Therefore, demonstrating the potential of transfersomas as a carrier of therapeutic agents, such as cannabidiol.

There are many studies done with cannabis-based extracts for pain in clinical trials. For example, Cannador® is a drug made of THC/CBD 2:1, used for spasticity changes in multiple sclerosis. It showed improvements, but had adverse effects such as sedation and psychoactive changes [2,49,50]. Another medication is Sativex®, an oral spray that has a partial agonist effect on CB1 and CB2. It features CBD, THC and other cannabinoids in its formula. Used in multiple sclerosis, cancer pain, and some types of chronic pain, showing significant improvement in pain reduction [2,51]. Therefore, studies have demonstrated promising results in the use of cannabidiol as an analgesic.

What can be highlighted in this research is the development and validation of an analytical method with precision and accuracy, using ultra-high performance liquid chromatography (UHPLC-MS). A simple and efficient method for identifying cannabidiol using only two solvents (water and ethanol) in the mobile phase without the need for changes in the proportion of solvents during the analysis run. Having an analytical run time of 3 minutes with CBD identified in the first minute.

We developed three new formulations containing cannabidiol (1%-2%) that had an average particle size of around 200 nm and the results of the tests on Franz cells showed that the TRANS formulation permeated the skin's cell layers more deeply than the CLN TRANS formulation, after 12 hours of experiment. The skin retention studies showed that the TRANS formulation reached, in greater quantities compared to CLN TRANS, the epidermis and dermis layers of the skin, after 12 hours of permeation. The likely reasons for these differences are the fact that TRANS is a formulation made with transferosomes, which are more malleable structures than the lipid nanocarriers present in the CLN TRANS formulation.

Searching for new topical therapeutic agents with fewer systemic side effects, non-invasive, and easy-to-apply, a study with patients was conducted using topical cannabidiol, which presented significant clinical results in pain relief (analgesia), probably acting on human endocannabinoid system. Despite the limitation of the number of patients, the results were promising [52].

Another study tested CBD delivery by transdermal to reduce pain and inflammation in rats that were induced to monoarthritis (knee joint inflammation). The results showed the potential of cannabidiol in the treatment of arthritic symptomatology [21]. Another research showed that cannabidiol applied transdermally to hairless guinea pigs led to the presence of CBD in the blood plasma, with transcutol® HP being an excellent enhancer of the drug permeation into the skin [53].

Studies carried out with cannabidiol (CBD) using Raman spectroscopy demonstrated two strong bands located in the range of 1664 cm^{-1}, attributed to ring 1, and 1644 cm^{-1}, attributed to isopropenyl C=C double bond stretchings. The Raman band at 1644 cm^{-1} is a characteristic vibrational frequency of the

CBD, another relevant region in the spectrum are the wavenumbers in the range from 1400 to 1250 cm^{-1} [54]. Another study presented data comparing experimental and simulated Raman spectra for cannabinoids, which demonstrated four predicted vibrational peaks at 1568, 1611, 1642 and 1683 cm^{-1} in the simulated CBD spectrum [55].

In studies carried out with Raman it was possible to identify that the peaks 1296 and 1440 cm^{-1} were a marker of the NLC and TRANS formulations at the Stratum corneum (SC) and epidermis (EP), respectively. The permeation profiles were determined by the intensity of this peaks for each time and each skin layers, and for each research subject. The area under de curve of the permeation profiles were calculated. The products presented different permeation dynamics on the stratum corneum and epidermis. The TRANS formulation keeps permeating to the end of epidermis for both times analyzed T4 and T8. The CLN TRANS formulation, tend to be retained at SC and EP for T4 and, most of the TRANS part of it looks like to be gone forward after 8 hours. However, this is a trend in theory, not being confirmed by the results obtained. Because the results presented a large standard deviation (dispersion), thus, they were not results with statistical significance.

Both products showed to permeate to the dermis, to the depth at 120 ± 5 μm. By comparing TRANS and CLN TRANS spectra to the dermis spectra, it was possible to identify that the peak 1662 cm^{-1} was a marker of the formulations' presence at the dermis. This peak is also known as the Amide I peak at the human dermis. Its intensity increased upon the use of TRANS and CLN TRANS at T4 and T8. The PCA analysis showed that both groups were formed when comparing T0 and T4, and that these groups were respective to the different times of analysis. Later, Loading Plots validated that the differences observed at the PCA analysis were related to the Amide I band (data not shown). Hence, a subtraction was performed to observe the net effect of the formulations. T4-T0 and T8-T0 showed that an increase of the Amide I band integrated area, between 1510 and 1720 cm^{-1} occurred for both formulations. The T4 and T8 net effect showed higher intensity for TRANS, in comparison to CLN TRANS. Also, intensities decreased between T4 and T8, leading to infer that at 120 ± 5 μm at T4, the amount of product was higher than at T8. In addition, the decrease between the net effect of T4 and T8 was much more accentuated for CLN TRANS. The results obtained with the most accurate studies in human skin confirmed what was observed in the *in vitro* tests. The study showed that the cannabidiol present in the TRANS formulation was in greater quantity, in dermis, after 8h of skin application when compared to CLN TRANS. Probably because the TRANS formulation has a more flexible vesicular structure and a greater ability to control the delivery of the encapsulated drug than the CLN TRANS.

CONCLUSION

The skin represents a more viable route for a cannabidiol delivery, especially for the presence of CB receptors. Thus, the topical administration of this drug is a more convenient, more tolerable therapeutic alternative, and with less side effects for pain relief. The challenge is to find a new formulation that is able to relieve the acute and chronic pain of patients, which includes rheumatoid arthritis, migraine, gout and other diseases. We demonstrate preliminary and viable studies using pork ear skin on cells Franz and Human Skin using Raman spectroscopy of a potential candidate for pain relief in patients. The data obtained in this study indicate that our formulations developed with cannabidiol have therapeutic

potential for topical use in the relief of acute and chronic pain, as cannabidiol was detected in the corneum stratum, epidermis and dermis of the skin, even identified in the receptor solution in the *in vitro* tests. Therefore, this is a promising field for cannabinoids, requiring further research studies to develop, improve and enable cannabidiol systems for topical and transdermal use commercially.

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REFERENCES

[1] R. Hudson, N. Puvanenthirarajah, Cannabis for pain management: Pariah or panacea? Historical perspectives and pharmacological mechanisms, UWOMJ, 87 (2018) 58.

[2] E. Russo, G.W. Guy, A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol, Med Hypotheses, 66 (2006) 234-246.

[3] T. Bisogno, L. Hanus, L Petrocellis, S Tchilibon, D.E Ponde, I. Brandi, A.S. Moriello, J.B. Davis, R. Mechoulam, V. Marzo, Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide, Br J Pharmacol., 134 (2001) 845-852.

[4] C.J.A. Morgan, H.V. Curran, Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis, Br. J. Psychiatry, 192 (2008) 306-307.

[5] L.R.M. Eagelston, N.K. Kalani, R.R Patel, H.K Flaten, C.A Dunnick, R.P Dellavalle, Cannabinoids in dermatology: a scoping review, Dermatol Online J., 24 (2018) 13030.

[6] N. Dmitrieva, H. Nagabukuro, D. Resuehr, G. Zhang, S.L. McAllister, K.A. McGinty, K. Mackie, K.J. Berkley, Endocannabinoid involvement in endometriosis, Pain., 151 (2010) 703-710.

[7] A.O. Tijani, D. Thakur, D. Mishra, D. Frempong, U.I. Chukwunyere, A. Puri, Delivering therapeutic cannabinoids via skin: Current state and future perspectives, J Control Release, 334 (2021) 427-451.

[8] I. Urits, K. Gress, K. Charipova, K. Habib, D. Lee, C. Lee, J.W. Jung, H. Kassem, E. Cornett, A. Paladini, G. Varrassi, A.D. Kaye, O. Viswanath, Use of cannabidiol (CBD) for the treatment of chronic pain, Best Pract Res Clin Anaesthesiol, 34 (2020) 463-477.

[9] N. Bruni, C.D. Pepa, S. Oliaro-Bosso, E. Pessione, D. Gastaldi, F. Dosio, Cannabinoid Delivery Systems for Pain and Inflammation Treatment, Molecules, 23 (2018) 2478.

[10] A.C. Howlett, C.S. Breivogel, S.R. Childers, S.A. Deadwyler, R.E. Hampson, L.J. Porrino, Cannabinoid physiology and pharmacology: 30 years of progress, Neuropharmacology, 47 (2004) 345-358.

[11] M.D.V Sickle, M. Duncan, P.J. Kingsley, A. Mouihate, P. Urbani, K. Mackie, N. Stella, A. Makriyannis, D. Piomelli, J.S. Davison, L.J. Marnett, V.D. Marzo, Q.J. Pittman, K.D. Patel, K.A., Sharkey, Identification and Functional Characterization of Brainstem Cannabinoid CB2 Receptors, Science, 310 (2005) 329-332.

[12] L. Chiou, S. S. Hu, Y. Ho, Targeting the cannabinoid system for pain relief?, Acta Anaesthesiol Taiwan, 51 (2013) 161-170.

[13] B.K. Atwood, K. Mackie, CB2: a cannabinoid receptor with an identity crisis, Br J Pharmacol, 160 (2010) 467-479.

[14] T. E. Sivesind, J. Maghfour, H. Rietcheck, K. Kamel, A.S. Malik, R.P. Dellavalle, Cannabinoids for the Treatment of Dermatologic Conditions, JID Innov., 2 (2022) 100095.

[15] C. J. Lucas, P. Galettis, J. Schneider, The pharmacokinetics and the pharmacodynamics of cannabinoids, Br J of Clin Pharmacol., 84 (2018) 2477-2482.

[16] A.W. Zuardi, History of cannabis as a medicine: a review, Braz. J. Psychiatry, 28 (2006) 153-157.

[17] A. Naik, Y.N. Kalia, R.H. Guy, Transdermal drug delivery: overcoming the skin's barrier function, Pharm Sci Technol Today, 3 (2000) 318-326.

[18] E. Keleb, R. Sharma, E.B. Mosa, A.Z. Aljahwi, Transdermal Drug Delivery System- Design and Evaluation, Int. J. of Advances in Pharm. Sci., 1 (2010) 201-211.

[19] A. Sharkawy, A.M. Silva, F. Rodrigues, F. Barreiro, A. Rodrigues, Pickering emulsions stabilized with chitosan/collagen peptides nanoparticles as green topical delivery vehicles for cannabidiol (CBD), Colloids Surf. A Physicochem. Eng. Asp., 631 (2021) 127677.

[20] C. Wang, B. Cui, Y. Sun, C. Wang, M. Guo, Preparation, stability, antioxidative property and in vitro release of cannabidiol (CBD) in zein-whey protein composite nanoparticles, LWT, 162 (2022) 113466.

[21] D.C. Hammell, L.P. Zhang, F. Ma, S.M. Abshire, S.L. McIlwrath, A.L. Stinchcomb, K.N. Westlund, Transdermal cannabidiol reduces inflammation and pain-related behaviours in a rat model of arthritis, Eur J Pain., 20 (2016) 936-948.

[22] A. Tubaro, A. Giangaspero, S. Sosa, R. Negri, G. Grassi, S. Casano, R. D. Loggia, G. Appendino, Comparative topical anti-inflammatory activity of cannabinoids and cannabivarins, Fitoterapia, 81 (2010) 816-819.

[23] M.P. Chelliah, Z. Zinn, P. Khuu, J.M.C. Teng, Self-initiated use of topical cannabidiol oil for epidermolysis bullosa, Pediatr Dermatol., 35 (2018) 224-227.

[24] S. Su, P.M. Kang, Recent Advances in Nanocarrier-Assisted Therapeutics Delivery Systems, Pharmaceutics, 12 (2020) 837.

[25] J. J. Escobar-Chávez, I.M. Rodríguez-Cruz, C.L. Domínguez-Delgado, R. Díaz-Torres, A.L. Revilla-Vázquez, N.C. Aléncaster, Nanocarrier Systems for Transdermal Drug Delivery, Intech, (2012) 201-240.

[26] J. Pardeike, A. Hommoss, R.H. Müller, Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products, Int J Pharm., 366 (2009) 170-184.

[27] E. B. Souto, J.F Fangueiro, A.R Fernandes, A. Cano, E. Sanchez-Lopez, M.L Garcia, P. Severino, M.O Paganelli, M.V Chaud, A.M Silva, Physicochemical and biopharmaceutical aspects influencing skin permeation and role of SLN and NLC for skin drug delivery, Heliyon, 8 (2022) e08938.

[28] V.G.S.S Jyothi, S. M. Ghouse, D. K. Khatri, S. Nanduri, S. B. Singh, J. Madan, Lipid nanoparticles in topical dermal drug delivery: Does chemistry of lipid persuade skin penetration?, J Drug Deliv Sci Technol., 69 (2022) 103176.

[29] E. Esposito, M. Drechsler, R. Cortesi, C. Nastruzzi, Encapsulation of cannabinoid drugs in nanostructured lipid carriers, Eur J Pharm Biopharm., 102 (2016) 87-91.

[30] M.W. Akram, H. Jamshaid, F.U. Rehman, M. Zaeem, J.Z. Khan, A. Zeb, Transfersomes: a Revolutionary Nanosystem for Efficient Transdermal Drug Delivery, AAPS PharmSciTech., 23 (2022).

[31] S.A.T. Opatha, V. Titapiwatanakun, R. Chutoprapat, Transfersomes: A Promising Nanoencapsulation Technique for Transdermal Drug Delivery, Pharmaceutics, 12(2020) 855.

[32] S. Rai, V. Pandey, G. Rai, Transfersomes as versatile and flexible nano-vesicular carriers in skin cancer therapy: the state of the art, Nano Rev Exp., 8 (2017) 1325708.

[33] K.B. Ita, J.D Preez, M.E. Lane, J. Hadgraft, J.D. Plessis, Dermal delivery of selected hydrophilic drugs from elastic liposomes: effect of phospholipid formulation and surfactants, J Pharma Pharmacol., 59 (2007) 1215-1222.

[34] G.M.E, Zaafarany, G.A.S Awad, S.M. Holayel, N.D. Mortada, Role of edge activators and surface charge in developing ultradeformable vesicles with enhanced skin delivery, Int J Pharm., 397(2010) 164-172.

[35] P. Chaurasiya, E. Ganju, N. Upmanyu, S. K. Ray, P. Jain, Transfersomes: a novel technique for transdermal drug delivery, J. drug deliv. ther., 9 (2019) 279-285.

[36] Li J., X. Wang, T. Zhang, C. Wang, Z. Huang, X. Luo, Y. Deng, A review on phospholipids and their main

applications in drug delivery systems, Asian J. Pharm. Sci., 10 (2015) 81-98.

[37] M. Badran, K. Shalaby, A. Al-Omrani, Influence of the Flexible Liposomes on the Skin Deposition of a Hydrophilic Model Drug, Carboxyfluorescein: Dependency on Their Composition, Sci. World J., 2012 (2012) 134876.

[38] G. Cevc, G. Blume, A. Schätzlein, Transfersomes-mediated transepidermal delivery improves the regio-specificity and biological activity of corticosteroids in vivo, J. Control. Release., 45 (1997) 211-226.

[39] G. Cevc, Transdermal drug delivery of insulin with ultradeformable carriers, Clin. Pharmacokinet., 42 (2003) 461-474.

[40] V. Garg, H. Singh, S. Bimbrawh, S.K. Singh, M. Gulati, Y. Vaidya, P. Kaur, Ethosomes and Transfersomes: Principles, Perspectives and Practices, Curr. Drug Deliv., 14 (2017) 613-633.

[41] L. Binder, S. SheikhRezaei, A. Baierl, L. Gruber, M. Wolzt, C. Valenta, Confocal Raman spectroscopy: In vivo measurement of physiological skin parameters - A pilot study, J. Dermatolol Sci., 88 (2017) 280-288.

[42] P.J. Caspers, G.W. Lucassen, E.A. Carter, H.A. Bruining, G. J. Puppels, In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles, J. Invest. Dermatol., 116 (2001) 434-442.

[43] C. Park, J. Zuo, V. Somayaji, B. Lee, R. Lobenberg, Development of a novel cannabinoid-loaded microemulsion towards an improved stability and transdermal delivery, Int. J. Pharm., 604 (2021) 120766.

[44] A. Sharkawy, F. Barreiro, A. Rodrigues, Pickering emulsions stabilized with chitosan/gum Arabic particles: Effect of chitosan degree of deacetylation on the physicochemical properties and cannabidiol (CBD) topical delivery, J. Mol. Liq., 355 (2022) 118993.

[45] V. Klang, J.C. Schwarz, B. Lenobel, M. Nadj, J. Auböck, M. Wolzt, C. Valenta, In vitro vs. in vivo tape stripping: validation of the porcine ear model and penetration assessment of novel sucrose stearate emulsions, Eur. J. Pharm. Biopharm., 80 (2012) 604-614.

[46] S. Demisli, E. Galani, M. Goulielmaki, F.L. Kyrilis, T. Ilic, F. Hamdi, M. Crevar, P.L. Kastritis, V. Pletsa, F. Nallet, S. Savic, A. Xenakis, V. Papadimitriou, Encapsulation of cannabidiol in oil-in-water nanoemulsions and nanoemulsion-filled hydrogels: A structure and biological assessment study, J. Colloid Interface Sci., 634 (2023) 300-313.

[47] S. Duangjit, P. Opanasopit, T. Rojanarata, T. Ngawhirunpat, Characterization and In Vitro Skin Permeation of Meloxicam-Loaded Liposomes versus Transfersomes, *J Drug Deliv.*, 2011 (2011) 418316.

[48] J. Shaji, M. Lal, PREPARATION, Optimization and evaluation of transferosomal formulation for enhanced transdermal delivery of a cox-2 inhibitor, Int. J. Pharm. Pharm. Sci., 6 (2014) 467-477.

[49] J. Zajicek, P. Fox, H. Sanders, D. Wright, J. Vickery, A. Nunn, A. Thompson, Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial, Lancet, 362 (2003) 1517-1526.

[50] A. Holdcroft, M. Maze, C. Doré, S. Tebbs, S. Thompson, A multicenter dose-escalation study of the analgesic and adverse effects of an oral cannabis extract (Cannador) for postoperative pain management, Anesthesiology, 104 (2006) 1040-1046.

[51] J.M. McPartland, E.B. Russo, Cannabis and Cannabis Extracts: Greater Than the Sum of Their Parts?, J Cannabis Ther., 1 (2001) 103-132.

[52] V. Maida, J. Corban, Topical Medical Cannabis: A New Treatment for Wound Pain-Three Cases of Pyoderma Gangrenosum, J Pain Symptom Manage., 54 (2017) 732-736.

[53] K.S. Paudel, D.C. Hammell, R.U Agu, S. Valiveti, A. L. Stinchcomb, Cannabidiol bioavailability after nasal and transdermal application: effect of permeation enhancers, Drug Dev Ind Pharm., 36 (2010) 1088-1097.

[54] G. Baranovic, Understanding the conformational, electronic and vibrational properties of Tetrahydrocannabinol (THC) and Cannabidiol (CBD). Pharmacophoric similarities and differences, J. Mol. Struct., 1244 (2021) 130945.

[55] T.J Wolfe, N. A. Kruse, M. M. Radwan, A. S. Wanas, K. N. Sigworth, M. A. ElSohly, N. I. Hammer, A study of major cannabinoids via Raman spectroscopy and density functional theory, Spectrochim. Acta A Mol. Biomol. Spectrosc., 303 (2023) 123133.