Nano-Suspensions of Preferred Cannabinoids

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ABSTRACT

In recent years, nano technology has been extensively investigated. Due to the submicron particle size and unique physicochemical properties of nano particles, they overcome the problems of low Cannabinoid's solubility and poor bioavailability. Although the structures of nano particles are simple, the further development of these materials is hindered by their stability. Cannabinoid's nano particles with the average sizes of 10 to 100 nm usually require the addition of stabilizers such as polymers or surfactants to enhance their stability. The stability of nano suspensions and the dispersibility of nano particles are the key factors for the large-scale production of formulations. In this paper, the factors that affect the stability of Cannabinoid's nano suspensions are discussed, and related methods for solving the stability problem is put forward. ***Methods:*** The Cannabinoid's formulation developed in this study consisted of vitamin E acetate, Cremphor L, Tween-60, and distilled water. ***Results:*** The particle size of the formulated suspension were in the range of 11.0 to 94 nm.

Introduction

Cannabinoids are chemical compounds found in the cannabis plant. There are more than 100 cannabinoids that have been isolated from cannabis[1]. Cannabidiol (CBD), tetrahydrocannabinol (THC) and cannabinol (CBN) are the most frequently studied species. Monitoring the cannabinoid composition of the extracts and purity of the isolated compounds is important to ensure the

potency of prepared formulations as well as proper sample handling in the laboratory. CBD has a similar chemical structure to that of THC, but it has no psychotropic activity[2-5]. CBD is known to modulate the activity of many cellular effectors including CB1 and CB2 receptors[3,6], 5HT1A receptors[7], GPR55[8], μ- and δ-opioid receptors[9], TRPV1 cation channels[10], PPARγ[11], and FAAH[10].

Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the primary active constituents of *Cannabis sativa*, with its isomer Δ^8-Tetrahydrocannabinol (Δ^8-THC) being a very minor component. Δ^9-THC has shown potential in the treatment of syndromes through its IOP lowering and neuroprotective effects[12,13]. The mechanism of action is not completely understood, though it has been said to have an agonistic action on the cannabinoid receptors CB_1 and CB_2[14,15]. These receptors are expressed on the iris-ciliary, retina-choroid and the trabecular meshwork[16]. Δ^9-THC, through these receptors, causes relaxation of the trabecular meshwork which results in increased aqueous humor drainage and subsequent IOP reduction[13]. Neuroprotective action of Δ^9-THC was also recently studied by El-Remessy et al. in N-methyl-D-aspartate (NMDA) induced retinal toxicity[13], making Δ^9-THC a promising candidate therapy. Previous reports from our group have demonstrated the physicochemical characteristics and permeability and *in vivo* disposition of Δ^9-THC and its relatively water-soluble prodrugs in the eye. The effects of ion pairing and micellar solutions on the disposition of Δ^9-THC in the eye were studied in these previous reports[17,18].

Cannabinol (CBN) is the nonenzymatic oxidation by-product of THC and is most commonly an artifact found after prolonged storage, especially at higher temperatures. CBN was the first cannabinoid to be identified and isolated from cannabis[19]. This discovery was

most likely due to rampant degradation of THC to CBN due to poor quality control, the transportation and storage conditions related to the 19th century; challenges that are still difficult to overcome in existing cannabis products[20]. A review of phytocannabinoids summarized the ability of CBN to inhibit the activity of a number of enzymes, including cyclooxygenase, lipoxygenase, and a host of cytochrome P450 (CYP) enzymes (e.g., CYP1A1, CYP1A2, CYP2B6, CYP2C9, CYP3A4, CYP3A5, CYP2A6, CYP2D6, CYP1B1, and CYP3A7)[21].

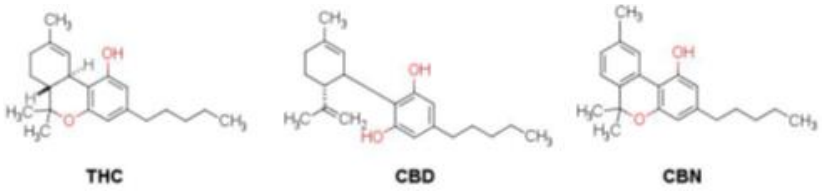

Figure 1: Cannabinoid Structures

Nanotechnologies

Nanotechnologies come with their own advantages and limitations. A best-in-class product designed with one nanotechnology does not guarantee the best-in-class formulation if that same technology is infused into another dosage form. Nanotechnology is an umbrella term used to define the products, processes, and properties at the nano-microscale that have resulted from the convergence of the physical, chemical, and life sciences. The National Nanotechnology Initiative (NNI) defines, "Nanotechnology as research and development at the atomic, molecular, or macromolecular levels in the sub-500-nm range (100 – 500 nm) to create structures, devices, and systems that have novel functional properties"[22].

Ultrasound assisted nanosuspension preparation

A vibrating ultrasonic probe immersed in a liquid will transmit alternating high- and low-pressure waves. These fluctuations cause the liquid molecular cohesive forces to break-down, pulling apart the liquid and creating millions of micro-bubbles (cavities), which expand during the low-pressure phases and implode violently during the high-pressure phases. As the bubbles collapse, millions of microscopic shock waves, micro jet streams, and eddies are generated at the implosion sites and propagated to the surrounding medium. Although this phenomenon, known as cavitation, lasts but a few microseconds, and the amount of energy released by each individual bubble is minimal, the cumulative amount of energy generated by the imploding cavities is extremely high promoting surface peeling, erosion, and particle breakdown. The volume of material that can be processed effectively with an ultrasonic processor is dependent on the power rating of the ultrasonic generator (power supply), and the diameter of the probe used with that power supply – the higher the rating of the power supply and the larger the diameter of the probe, the larger the volume of material which can be processed. The equipment of our choice for processing batches is Branson 550 model with a solid probe purchased from Fisher Scientific (Toronto, Canada).

Materials And Methods

Chemicals and Reagents

Phyto cannabinoid analytical standards (> 98%) Δ^9-THC, Δ^8-THC CBD, and CBN were purchased from Millipore Sigma (Toronto, Canada). Liquid chromatography (LC) grade acetonitrile, methanol, formic acid and water for the mobile phase and sample preparation were purchased from Fisher Scientific (Toronto, Canada).

Preparation of nanosuspensions

Low concentrated (0.5 to 2 mg/ml) nano-suspension of a preferred cannabinoid preparation process.

Process of the nano suspension formulation comprise of:

a. Base preparation
b. Adding preferred cannabinoids
c. Adding Aqueous Medium (deionized H_20)

Base preparation comprises of mixing carrier oil, D-α Tocopherol and Cremophor EL. There are two options what to use as the carrier oil. First option is the cold pressed hemp oil. It has few advantages and the main one is the similarities to cannabinoids. Also, few disadvantages and the main one is the limitation. According to the health regulations, daily consumption should not be more than 7g/kg (for average human of 70kg). Second option is the corn oil. Absolute advantage is the easy to digest by human metabolism.

Thus, in clean bicker (200g) 10g of a carrier oil (of choice) was added, and then added 5 g of D-α Tocopherol and 50 g of Cremophor EL was added. Components were mixed together and when mix becomes clear 195g of Tween 60 was added. Resulting base was heated to 60°C and 0.5 to 2 g. of the preferred cannabinoid was added. Resulting mix was stirred slowly until absolutely clear and then transferred to 2000 ml clean bicker. Bicker with the mix was heated to 60°C in a water bath. Ultrasound mixing was applying for 3 minutes in order to minimize foam creation. Resulting nano suspension was cooled and average size of the droplets was performed.

High concentrated (10 to 500 mg/ml) nano-suspension of a preferred cannabinoid preparation process.

Thus, in clean bicker (200g) 10g of a carrier oil (of choice) was added, and then added 5 g of D-α Tocopherol and 50 g of Cremophor EL was added. Components were mixed together and when mix becomes clear 195g of Tween 80 was added. In order to prepare high concentrated nano-suspension the base content must be adjusted accordingly to the cannabinoid concentration. Appropriate resulting mix was stirred slowly until absolutely clear and then transferred to 2000 ml clean bicker. Bicker with the mix was heated to 60°C in a water bath. Ultrasound mixing was applying for 3 minutes in order to minimize foam creation. Resulting nano suspension was cooled and average size of the droplets was performed.

Chemical Analysis

Δ^9-THC, Δ^8-THC CBD, and CBN were analyzed by UHPLC/UV (Shimadzu SIL 40C XS).

METHOD INFO

Column: XBridge C-18; 5µm; 4.6x150mm; Internal Column Number:#59

Mobile Phase: A – 0.1% Formic Acid in H_2O; B - 0.05% Formic Acid in Methanol (HPLC Grade)

Time Table:

Minute	A%	B%
0.0	40.0	60.0
7.0	23.0	77.0
8.2	5.0	95.0
14.0	40.0	60.0

Stop Time: 18.0 Min.;

Post Time: 2.0 min;

Flow: 1.0 ml/min;

Injection volume: 10µL;

Detection UV: A – 210nm, B – 230nm;

Mean Particle Size and Particle-Size Distribution

The mean particle size and the width of particle-size distribution are important characterization parameters as they govern the saturation solubility, dissolution velocity, physical stability and even biological performance of nanosuspensions. Mean particle size distribution was measured by Anton Paar Litesizer Model 500. Diluted nanosuspension was added to the sample cell (quartz cuvette) and put into sample holder unit and measurement was carried out with help of software. Zeta potential of the optimized formulation was measured using the same instrument. Sample was added in specialized zeta cell and the same procedure was carried out as described earlier. Large magnitude values of zeta potential indicate higher stability of a suspension. For the purpose of determining the stability of prepared nanosuspension imparted by various stabilizers, the zeta potential of all the samples was measured. All the zeta potential measurements of nanosuspensions containing different stabilizers were carried out in triplicate with the mean values and standard deviations reported.

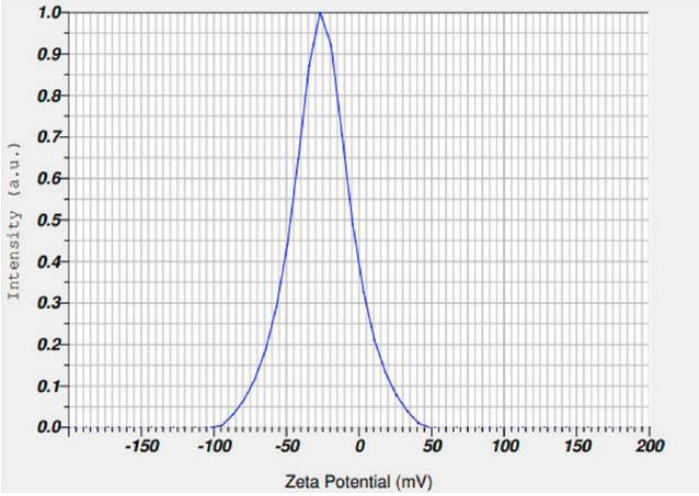

Fig.2. Zeta potential of the nanosuspension

Stability Study

Three batches, each of nanosuspension and lyophilized nanosuspension were used for each storage condition. At periodic

time intervals, the samples were withdrawn and analyzed for particle size and drug content.

Saturation solubility determination

Saturation solubility was assessed for both unprocessed pure drug and optimized nanosuspension. Accurately weighed 10 mg of pure cannabinoid and nanosuspension equivalent to 10 mg of it was separately introduced into a 25 ml stoppered conical flask containing 10 ml of distilled water. The sealed flask was placed in a rotary shaker at 37°C and equilibrated for 6 hours. The contents were then filtered, and the suitably diluted samples were analyzed using UV-visible spectrophotometer (Shimadzu-1900, Japan) at 285 nm, against distilled water as a blank. Each sample was prepared and analyzed in triplicate.

Short-term physical stability

Physical stability of the optimized nanosuspension for partially open vial was evaluated for a period of up to 1 month at ambient conditions (22°C). Nanosuspension was stored in a closed standard polymer cuvette provided by Anton Paar. The stability was assessed in terms of PSD, zeta potential, total weight and percent drug loading.

Drug loading

The amount of cannabinoid present as nano-sized drug particle in the dispersion was measured by UV-visible spectroscopy. Drug loading of cannabinoid in nanosuspension was found to be in the range of 15%-81%. Larger particles that were not in the submicron range must have settled down at the bottom, leading to low drug loading in various formulations of the nanosuspension.

Results

Quantification of Cannabinoids Using HPLC

Linear calibration curves for Cannabinoids were obtained at ranges of 10–200. Their correlation coefficients (R^2) were

between 0.985 and 0.998. The lower limit of detection was 10 ng/mL. In the intra-day analysis, precision and accuracy were 15.8% at 10 ng/mL and 3.58% at 200 ng/mL, respectively. In the inter-day analysis, precision and accuracy were 8.20% at 10 ng/mL and 2.88% at 100 ng/mL, respectively. These results satisfied the acceptance criteria (< 20 and 15% for the lower and higher concentrations, respectively).

Characteristics of nano suspensions

FIG. 1 shows the images taken by observing the appearance of each of the nanosuspension formulations (when viewed from the left side, CBD, Δ^9-THC, Δ^8-THC, and CBN). The mean particle size of transparent formulation immediately after the preparation, was determined to be 35.3 ± 1.8 nm (*n* = 5), for CBD; 22.0 ± 1.8 nm (*n* = 5) for Δ^9-THC, 22.0 ± 1.8 nm (*n* = 5) for Δ^9-THC and 44.5 ± 1.8 nm (*n* = 5) for CBN, respectively.

Discussion

Nano-cannabinoids are to provide exceptionally high bioavailability and therapeutic effect, and are absorbed by the body, either orally or through the skin, very rapidly and completely. In fact, the uptake starts to occur in the mouth almost immediately upon oral administration. This means higher potency and faster onset of action for lower doses. In addition, nano-cannabinoids are water-compatible and can be easily mixed into beverages at essentially any desired concentration. To improve the absorption and bioavailability of cannabinoids, we developed a new nano sized formulation[23-25]. The appearance and particle diameter of nanosuspensions were the same during storage at 22°C./60% relative humidity (RH) for at least 18 months (Nitrogen filled, closed vials and not exposed to a direct light). The appearance of the nanosuspensions preparation developed in this study was transparent and its particle

diameter was small enough to be evaluated as Nano (< 45 nm as the mean value). The formulations solubilized and remained transparent even after extensive dilution with water (e.g., > 100 times diluted), indicating that this were a self-emulsifying system. The choice and concentration of stabilizer are selected to promote the particle size reduction process and generate physically stable formulations. To be effective, the stabilizer must be able to wet the surface of the drug and providing a steric or ionic barrier. In the absence of the appropriate stabilizer, the high surface energy of nanometer sized particles would tend to agglomerate or aggregate the drug. Physically stable nano formulations are obtained when the weight ratio of drug to stabilizer is 20:1 to 2:1. Too little stabilizer induces agglomeration or aggregation and too much stabilizer promotes Ostwald ripening.

The process of identifying an appropriate stabilizer(s) for a drug candidate is empirical and can be accomplished using amount of drug in milligram. Pharmaceutical excipients such as the polysorbates, cellulosic, povidones and plutonic are usually used that are acceptable stabilizers for creating physically stable nanoparticle dispersions. NCs are noticeably easy to prepare, but then again, the stability and the selection of stabilizer(s) greatest challenging and critical step[27]. Advancement of nanosized particles makes high energy surfaces, which can spin to aggregate and Ostwald ripening, if stabilization isn't at an efficient level. Stabilizers can be non-ionic or ionic in nature and the general steadiness depends on the established DLVO-hypothesis came to either by means of electrostatic powers or steric block.

1. Polymers: HPMC, HPC, MC[28,29], PVP[30], poloxamers[30,31]
2. Nonionic: Polysorbates, Sorbitan esters, vitamin E TPGS[32,33].
3. Ionic: SDS[29].

In this study, we used several ingredients, i.e., water, Cremphor L, Tween-60, and vitamin E acetate (D-α Tocopherol) to emulsify cannabinoids for nanosuspensions. MCT Oil was used as a co-solvent to dissolve cannabinoids of our choice in the lipid phase, which was also used for Neoral®[26], the commercially available microemulsion formulation of cyclosporine A. Tween-60, which was used as a surfactant, is generally known to be a non-toxic and non-irritant material[34,35]. However, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended the acceptable daily intake of Tween-60 without any side effects to be 25 mg/kg body weight.

Decrease in particle size to nano range results in improved saturation solubility as well as dissolution velocity[36,37].

The relation between the saturation solubility of a drug and its particle size is inversely proportional to each other (according) to which decrease in particle size results in increase in Surface area consequently saturation solubility of the drug[38]. Nanosuspension offers large surface area which increase the contact area of each particle and solvent system which tremendously increase the passing of drug from bulk to solution hence dissolution velocity is fastened[39,40]. This relationship can be expressed by simple Noyes Whitney equation:

$$\frac{dC}{dt} = \frac{DS}{h}\left(C_s - C_t\right)$$

Where dC/dt is the rate of dissolution, D is the diffusion coefficient of a drug in solution, S is the effective surface area of a drug in contact with the fluid, C_s is the saturation solubility of the drug in the diffusion layer, C_t is the concentration of the drug in the bulk medium and h is the diffusional distance over which the concentration gradient happens.

The drop of particle size, increased saturation solubility, an enlarged surface area and a thinner diffusion layer can intensely increase the dissolution velocity, which ultimately improves bioavailability of drug in body[41]. Therefore, reduction in particle size is a good approach to successfully improve the drug bioavailability where the drug's dissolution speed is the rate limiting step[42]. Via moving to nanonization from micronization, the rate of dissolution increases because of particle shell is further increase. In many cases, a low dissolution speed is related to low saturation solubility[43] but by enhancing the saturation solubility concentration gradient between the blood and gut lumen, then the absorption by passive diffusion[44]. Like other nanoparticles, nanosuspensions showed up an increased or improved adhesiveness to tissue which generally led to an enhancement in oral absorption of poorly soluble drugs apart from the increased dissolution rate and saturation solubility[45,46]. It is well known that amorphous drugs possess a higher saturation solubility than crystalline drug material. Amorphous drug nanoparticles possess a higher saturation solubility as compared to equally sized drug in the crystalline state. Therefore, to achieve the highest saturation solubility[47], a combination of nanometer size and amorphous state is ideal.

The formulations developed all comprised surfactant (Cremophor or Labrasol, at 20% w/w), a separate oil phase, (a number of oils were tested which were proprietary forms of: glycerol monocaprylocaprate, caprylic/capric triglyceride, propylene glycol caprylate and propylene glycol dicaprylate/dicaprate were tested, typically at 4% w/w), and a co-surfactant (including Transcutol, PEG 400, glycerol, ethanol and propylene glycol, typically at concentrations between 20 and 35% w/w).

For producing clear suspensions, the majority of droplets fall below 100 nm to create minimum turbidity. For nano-suspensions, the dimensions of the particulate phase are much smaller than the wavelength of light, causing weak light scattering and hence low turbidity. Therefore, nano-suspension tend to be transparent in appearance (Fig.1).

The stable formulations are subjected to a stability test at 40°C., at room temperature (22°C) and under the condition of storage under cooling (4°C). To determine the stability of a formulation, each formulation was stored in a transparent container in a thermostat at 40° C., at room temperature (22°C) and under cooling (4°C), and the stability is evaluated by the naked eyes at a predetermined time interval and observing the separation or precipitation in the formulation. In addition, during the test of formulation stability, the stability is determined by average size of the droplets for each formulation. Average size measurement reports are shown in Table 1.

Table 1. Average size measurement results.

Concentration of Cannabinoid (mg/ml)	Date of creation	Size at the initial date (nm)	Size at January 7 2022 (nm)
0.5	06 Mar 2019	10.1	11.08
1.0	09 May 2019	11.0	12.67
2.0	09 Nov 2019	12.7	15.35
5.0	09 Nov 2019	15.4	17.92
10.0	09 Nov 2019	54.91	63.09
125.0	09 Nov 2019	92.48	112.89
500.0	09 Nov 2019	720.6	980.67

It can be seen that the nano-emulsions including high concentrated APC are stable for the period of more than 36 month. In certain cases, agglomeration of nano-suspension may occur. In this regard, proper dispersants may be employed to minimize agglomeration, alone or in combination with

surfactants. Suitable co-solvents include, but are not limited to, transcutol, PEG 300-8000, glycerol, and ethanol[48,49].

Short-term physical stability

The optimized nanosuspension formulation showed good physical stability. 1 ml of the optimized nanosuspension was placed into a standard polymer cuvette and total weight was recorded. All nanosuspensions were created at 19 Oct 2020. The results of the stability studies are shown in Table 2.

Table 2. Average size measurement results.

Nanosuspension of Cannabinoid at 10mg/ml	Initial total weight (g)	Size at the initial date (nm)	Size at January 7 2022 (nm)
CBD	3.26	19.88	37.69
Δ^9-THC	3.501	83.12	94.09
Δ^8-THC	3.808	37.37	76.19
CBN	3.428	18.52	33.03

The results showed that temperature has an influence on aggregation of nanoparticles and at room temperature, aggregation was higher compared to closed vials storage condition for liquid nanosuspension. When comparing liquid nanosuspension with lyophilized nanosuspension, aggregation was more in liquid state for both conditions. Refrigerated condition has no significant effect on particle size whereas room temperature condition has more detrimental effect. The conclusion is that higher temperature results in increase in particle size. The effect is more significant in liquid nanosuspension as compared to dry formulation. The increase in the particle size at room temperature is thought to be due to the aggregation of the particles. Another reason may be the Ostwald ripening resulting from fluctuations in room temperature.

Fig.3 – 5 show the total weight decrease for semi-closed vials storage condition at room temperature

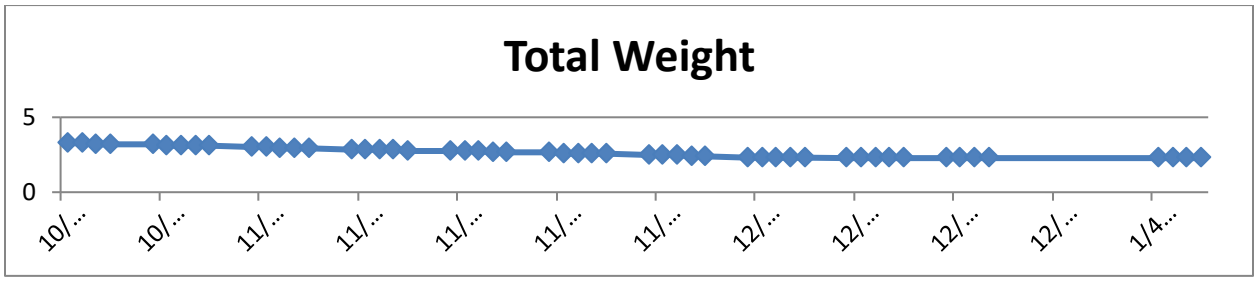

Fig.3 Total weight of CBD nanosuspension

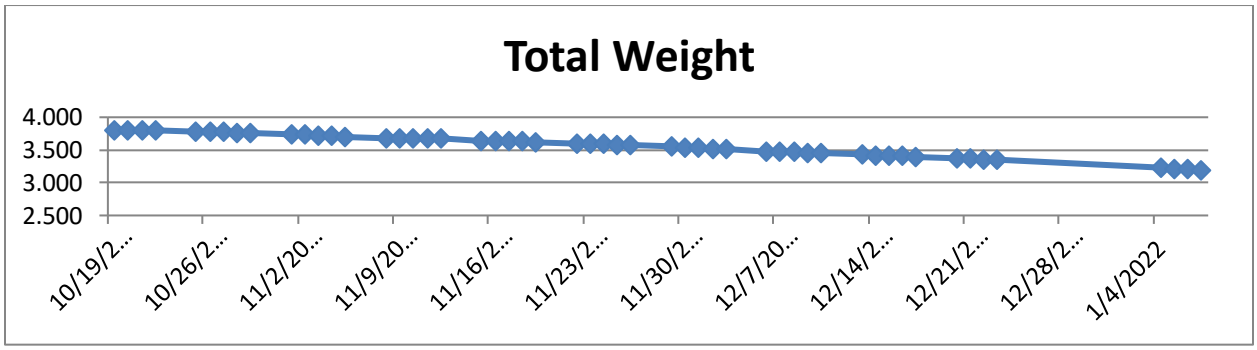

Fig.4 Total weight of Δ^9-THC nanosuspension

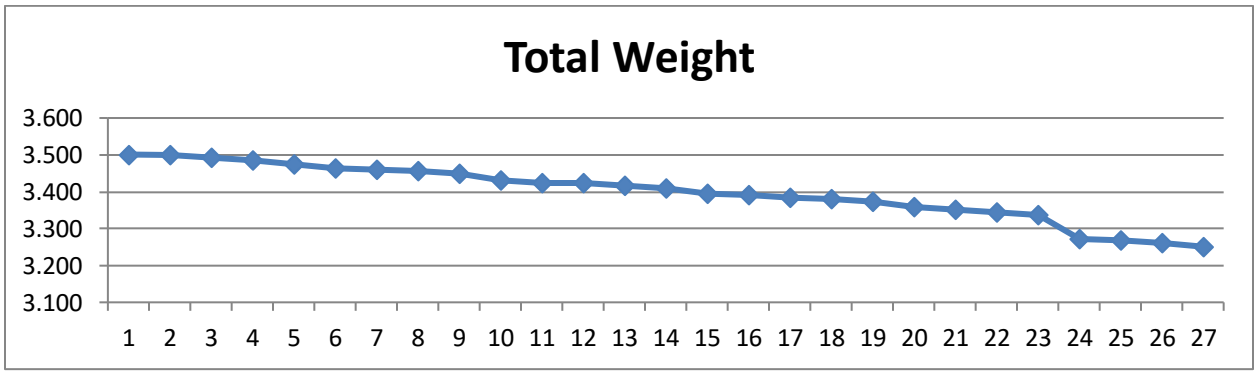

Fig.4 Total weight of Δ^8-THC nanosuspension

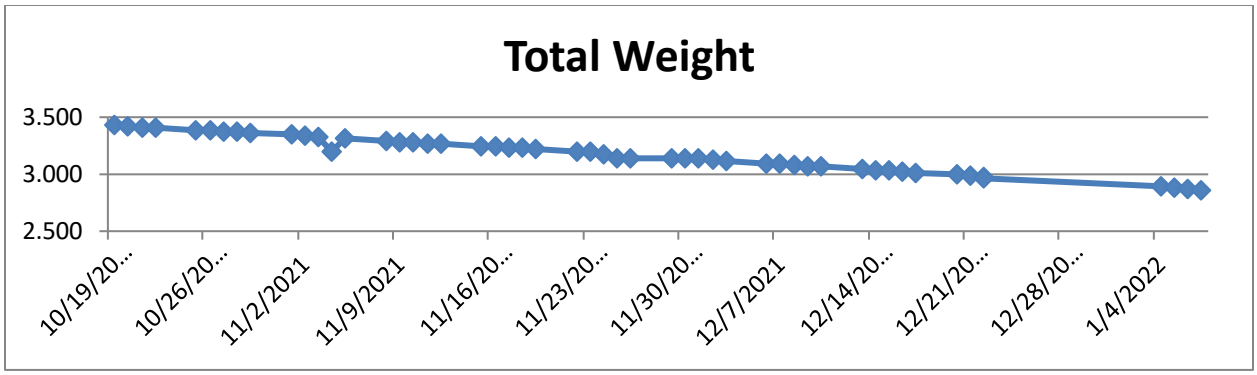

Fig.4 Total weight of CBN nanosuspension

CONCLUSION

Nanosuspension of preferred cannabinoid was successfully prepared using ultrasound homogenization as a wet milling technique. Preliminary investigative studies and evaluation of critical parameters like zeta potential, particle size distribution, and drug loading indicate that the combination of presented nano carrier and preferred cannabinoid exhibits a narrow range of size distribution of nanosuspension with a narrow particle size distribution and a stable zeta potential value −25.8 mV. All nanosuspension demonstrated prolonged particle size retention without agglomeration. This phenomena might be explained by electro steric stabilization of the nanosuspension due to higher electrostatic repulsive forces between the particles, as well as enhanced steric hindrance from the adsorbed polymer. As the effect of stabilizer type and ratio used provided an important direction for optimization of formulation parameters with respect to the mean particle size, drug loading, and zeta potential as evident from the results. Furthermore, the findings from envisaged research suggest ultrasonic homogenization as a reasonable, novel, and alternative top-down approach for the production of stable nanosuspension of preferred cannabinoids. However, further

studies with suitable tools, like design of experiment, are imperative in order to explore the formulation and process variables minutely.

CONFLICT OF INTEREST

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

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None.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

ETHICAL APPROVAL

Not applicable.

References

1. Aizpurua-Olaizola, O. *et al.* Evolution of the Cannabinoid and Terpene Content during the Growth of Cannabis sativa Plants from Different Chemotypes. *J. Nat. Prod.* **79**,324–331 (2016).
2. McPartland JM, Duncan M, Di Marzo V, Pertwee RG. Are cannabidiol and Δ(9)-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br J Pharmacol. 2015 Feb; 172(3): 737–53.

3. Rong C, Lee Y, Carmona NE, Cha DS, Ragguett RM, Rosenblat JD, et al. Cannabidiol in medical marijuana: research vistas and potential opportunities. Pharmacol Res. 2017 Jul; 121: 213–8.
4. Ibeas Bih C, Chen T, Nunn AV, Bazelot M, Dallas M, Whalley BJ. Molecular targets of cannabidiol in neurological disorders. Neurotherapeutics. 2015 Oct; 12(4): 699–730.
5. Iffland K, Grotenhermen F. An update on safety and side effects of cannabidiol: a review of clinical data and relevant animal studies. Cannabis Cannabinoid Res. 2017 Jun; 2(1): 139–54.
6. Hayakawa K, Mishima K, Hazekawa M, Sano K, Irie K, Orito K, et al. Cannabidiol potentiates pharmacological effects of Delta(9)-tetrahydrocannabinol via CB(1) receptor-dependent mechanism. Brain Res. 2008 Jan; 1188: 57–64.
7. Russo EB, Burnett A, Hall B, Parker KK. Agonistic properties of cannabidiol at 5-HT1a receptors. Neurochem Res. 2005 Aug; 30(8): 1037–43.
8. Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol. 2007 Dec; 152(7): 1092–101.
9. Kathmann M, Flau K, Redmer A, Trankle C, Schlicker E. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. Naunyn Schmiedebergs Arch Pharmacol. 2006 Feb; 372(5): 354–61.
10. Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, et al. 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. 2001 Oct; 134(4): 845–52.

11. Campos AC, Moreira FA, Gomes FV, Del Bel EA, Guimaraes FS. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. Philos Trans R Soc Lond B Biol Sci. 2012 Dec; 367(1607): 3364–78. in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. Philos Trans R Soc Lond B Biol Sci. 2012 Dec; 367(1607): 3364–78.
12. Crandall J, Matragoon S, Khalifa YM, Borlongan C, Tsai NT, Caldwell RB, et al. Neuroprotective and intraocular pressure-lowering effects of (-)Delta9-tetrahydrocannabinol in a rat model of glaucoma. Ophthalmic Res. 2007;39:69–75.
13. El-Remessy AB, Khalil IE, Matragoon S, Abou-Mohamed G, Tsai NJ, Roon P, et al. Neuroprotective effect of (-)Delta9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. Am J Pathol. 2003;163:1997–2008.
14. He F, Song ZH. Molecular and cellular changes induced by the activation of CB2 cannabinoid receptors in trabecular meshwork cells. Mol Vis. 2007;13:1348–56
15. Njie YF, Qiao Z, Xiao Z, Wang W, Song ZH. N-arachidonylethanolamide-induced increase in aqueous humor outflow facility. Invest Ophthalmol Vis Sci. 2008;49:4528-34.
16. Porcella A, Maxia C, Gessa GL, Pani L. The human eye expresses high levels of CB1 cannabinoid receptor mRNA and protein. Eur J Neurosci. 2000;12:1123–7.
17. Hingorani T, Adelli GR, Punyamurthula N, Gul W, Elsohly MA, Repka MA, et al. Ocular disposition of the hemiglutarate ester prodrug of (9)-Tetrahydrocannabinol from various ophthalmic formulations. Pharm Res. 2013;30:2146–56.
18. Hingorani T, Gul W, Elsohly M, Repka MA, Majumdar S. Effect of ion pairing on in vitro transcorneal permeability of a

Delta(9) -tetrahydrocannabinol prodrug: potential in glaucoma therapy. J Pharm Sci. 2012;101:616–26.

19. Wood, T. B., Spivey, W. T. N., & Easterfield, T. H. (1899). III. Cannabinol. Part I. Journal of the Chemical Society, Transactions, 75, 20-36.
20. Upton, R., Craker, L., ElSohly, M., Romm, A., Russo, E., & Sexton, M. (2013). Cannabis inflorescence: Cannabis spp.: Standards of identity, analysis and quality control. In R. Upton (Ed.), Scotts Valley, CA, USA: American Herbal Pharmacopoeia.
21. Pertwee, R. G., & Cascio, M. G. (2014). Known pharmacological actions of delta-9-tetrahydrocannabinol and of four other chemical constituents of cannabis that activate cannabinoid receptors. In R. G. Pertwee (Ed.), Handbook of cannabis (pp. 115–136). Oxford, UK: Oxford University Press.
22. K. J. Morrow, R. Bawa, and C. Wei, "Recent advances in basic and clinical nanomedicine," *Medical Clinics of North America*, vol. 91, no. 5, pp. 805–843, 2007.
23. Heimbach T, Fleisher D, Kaddoumi A. Overcoming poor aqueous solubility of drugs for oral delivery. In: Prodrugs: Springer New York; 2007. p. 157-215.
24. Alam MA, Al-Jenoobi FI, Al-mohizea AM. Commercially bioavailable proprietary technologies and their marketed products. Drug Discov Today 2013; 18(19-20): 936-49.
25. Rabinow BE. Nanosuspensions in drug delivery. Nat Rev Drug Discov 2004; 3(9): 785-96.
26. Narang AS, Delmarre D, Gao D. Stable drug encapsulation in micelles and microemulsions. Int J Pharm. 2007 Dec; 345(1-2): 9-25.
27. Peltonen L, Hirvonen J. Pharmaceutical nanocrystals by nanomilling: critical process parameters, particle

fracturing and stabilization methods. J Pharm Pharmacol 2010; 62(11): 1569-79.

28. Tuomela A, Liu P, Puranen J, et al. Brinzolamide nanocrystal formulations for ophthalmic delivery: reduction of elevated intraocular pressure in vivo. Int J Pharm 2014; 467(1-2): 34-41.
29. Gaunia Anju, Mazumder R, Pathak K. Formulation, optimization and characterization of ziprasidone nanocrystals prepared by media milling technique. Int J Pharm Pharm Sci 2015; 7(8): 146-50.
30. Liu P, Viitala T, Kartal-Hodzic A, et al. Interaction studies between indomethacin nanocrystals and peo/ppo copolymer stabilizers. Pharm Res 2015; 32(2):628-39.
31. Liu P, Rong X, Laru J, et al. Nanosuspensions of poorly soluble drugs: preparation and development by wet milling. Int J Pharm 2011; 411(1-2): 215-22.
32. Van Eerdenbrugh B, Vermant J, Martens JA, et al. A screening study of surface stabilization during the production of drug nanocrystals. J Pharm Sci 2009;98(6): 2091-103.
33. Ahuja BK, Jena SK, Paidi SK, Bagri S, Suresh S. Formulation, optimization and in vitro-in vivo evaluation of febuxostat nanosuspension. Int J Pharm 2015; 478(2): 540-52
34. Seeballuck F, Lawless E, Ashford MB,O'Driscoll CM. Stimulation of triglyceriderich lipoprotein secretion by polysorbate 80: in vitro and in vivo correlation using Caco-2 cells and a cannulated rat intestinal lymphatic model. Pharm Res. 2004 Dec; 21(12): 2320-6.
35. Kaur G, Mehta SK. Developments of Polysorbate (Tween) based microemulsions: preclinical drug delivery, toxicity and

antimicrobial applications. Int J Pharm. 2017 Aug; 529(1-2):134-60.

36. Laaksonen T, Limnell T, Santos H, et al. Drug dissolution studies on mesoporous silicon particles-a theoretical approach. Eur J Pharm Sci 2008; 34(1): S35.
37. Zhou Y, Du J, Wang L, Wang Y. Nanocrystals technology for improving bioavailability of poorly soluble drugs: a mini-review. J Nanosci Nanotechnol 2017; 17(1): 18-28.
38. Kesisoglou F, Panmai S, Wu Y. Nanosizing - Oral formulation development and biopharmaceutical evaluation. Adv Drug Deliv Rev 2007; 59(7): 631-44.
39. Mauludin R, Müller RH, Keck CM. Kinetic solubility and dissolution velocity of rutin nanocrystals. Eur J Pharm Sci 2009; 36(4-5): 502-10.
40. Junyaprasert VB, Morakul B. Nanocrystals for enhancement of oral bioavailability of poorly watersoluble drugs. Asian J Pharm Sci 2015; 10(1): 13-23.
41. Guo S, Huang L. Nanoparticles containing insoluble drug for cancer therapy. Biotechnol Adv 2014; 32(4): 778-88.
42. Salazar J, Muller RH, Moschwitzer JP. Performance comparison of two novel combinative particle-sizereduction technologies. J Pharm Sci 2013; 102(5): 1636-49.
43. Armijo LM, Brandt YI, Withers NJ, et al. Multifunctional superparamagnetic nanocrystals for imaging and targeted drug delivery to the lung. In: Parak WJ, Yamamoto K, Osinski M, Eds. Colloidal Nanocrystals for Biomedical Applications. Bellingham: International Society for Optics and Photonics 2012; pp. 82320M.
44. Gao L, Zhang D, Chen M. Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. J Nanoparticle Res 2008; 10(5): 845-62.

45. Möschwitzer J, Müller R. Drug nanocrystals-the universal formulation approach for poorly soluble drugs. In: Thassu D, Deleers M, Pathak YV, Eds. Nanoparticulate drug delivery systems. CRC Press: Florida 2007; pp. 71-88.
46. Ponchel G, Montisci M-J, Dembri A, Durrer C, Duchêne D. Mucoadhesion of colloidal particulate systems in the gastro-intestinal tract. Eur J Pharm Biopharm 1997; 44(1): 25-31.
47. Colombo M, Staufenbiel S, Rühl E, Bodmeier R. In situ determination of the saturation solubility of nanocrystals of poorly soluble drugs for dermal application. Int J Pharm 2017; 521(1-2): 156-66.
48. Jacobs C, Kayser O, Müller RH. Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide. Int J Pharm 2000; 196(2): 161-4.
49. Müller RH, Jacobs C. Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability. Int J Pharm 2002; 237(1-2): 151-61.
50. "Basic Report: 12012, Seeds, hemp seed, hulled". USDA National Nutrient Database. April 2018. Archived from the original on February 22, 2019. Retrieved February 22, 2019.
51. USDA National Nutrient Database, Standard Release 21. 2014. Retrieved September 7, 2017. Values from Nutritiondata.com (SR 21) may need to be reconciled with most recent release from the USDA SR 28 as of Sept 2017.
52. "US National Nutrient Database, Release 28". United States Department of Agriculture. May 2016
53. US National Nutrient Database, Release 28, United States Department of Agriculture. May 2016. Retrieved 6 September 2017.