A clinical investigation into the bioavailability of a *peroral* cannabinoid-Pheroid® formulation

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This Master's dissertation is dedicated to my loving maternal grandparents:

Koos Bodenstein, who has always been enthusiastic and passionate about science and greatly influenced me to pursue research & **Ansie Bodenstein**, who (despite her inspiring strength) would have benefitted immensely from an enhanced and approved pure Cannabidiol product in her battle lost against MS.

~

A journey of a thousand miles begins with a single step – Confucius

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PREFACE

I, Stephanie Erasmus, declare that the dissertation entitled 'A clinical investigation into the bioavailability of a *peroral* cannabinoid-Pheroid® formulation' which I hereby submit to the North-West University is in compliance of the Master of Sciences in Pharmaceutical Science degree, is my own work and has not yet been submitted to any other university.

I would like to acknowledge the following persons and establishments for their contributions during this study:

- Dr. John Takyi-Williams developed and validated the LC-MS/MS method used during the study. He performed sample preparations and the LC-MS/MS analyses of all collected blood samples.
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- The MooiMed Private Hospital, Potchefstroom, was used as clinical trial study site during the study arms. The MooiMed personnel assisted during the study initiation and study arms.
- The Research Institute for Industrial Pharmacy, incorporating Centre for Quality Assurance Medicines (RIIP®/CENQAM®) provided the stability chambers and performed HPLC analysis.
- Financial assistance was provided by the Pheroid® Cluster and the North-West University.

ABSTRACT

The therapeutic advantages of pure Cannabidiol (CBD), a phytocannabinoid isolated from the *Cannabis sativa* L. plant, are emphasised by the growing use of CBD in numerous conditions ranging from supplementary to the treatment of severe and debilitating forms of epilepsy. The wide spectrum of therapeutic effects associated with pure CBD is achieved without serious adverse events; providing a rationale for chronic CBD use in these conditions. However, the bioavailability of CBD is limited during oral administration due to the inherently high lipophilic nature of cannabinoids. Pheroid®, an effective and well-established drug delivery system, is known to increase bioavailability of pharmaceutical products. The Pheroid® was formulated to potentially improve the drug properties, with the focus on drug bioavailability. A CBD-pro-Pheroid® drug was developed and optimised. The optimised drug (together with a pro-Pheroid® control) was subjected to 6 months' accelerated stability testing at conditions 5°C, 25°C/60% RH, 30°C/70% RH and 40°C/75% RH. Characterisation analyses performed included morphology analysis, particle size distribution and zeta potential measurements. The formulation pH, capsule integrity and CBD concentrations were also measured to obtain a comprehensive profile of the CBD-pro-Pheroid® formulation. Preliminary study results confirmed that the optimized CBD-pro-Pheroid® was stable when kept at 25°C/60% RH for 6 months. The stability results also emphasised the need for an established method to formulate and confirm the quality of commercially available and artisanal CBD-preparations.

The main objective of the study was to evaluate the bioavailability of the optimised CBD-pro-Pheroid® formulation. This was achieved by conducting a phase 1 clinical trial. The CBD-pro-Pheroid® formulation (containing 20 mg pure pharmaceutical grade CBD and 450 mg pro-Pheroid®) was orally administered as a test formulation and pure CBD was administered as a reference compound to 14 healthy participants. Both male and female participants were included in the randomised cross-over, single-dose and single-centre clinical trial. Following the oral administration of the CBD-pro-Pheroid® consecutive blood samples were collected for 48 hours to obtain a complete pharmacokinetic profile of the formulation. The pharmacokinetic values, including the Area under the curve as a function of bioavailability, and the safety associated with the optimised CBD-pro-Pheroid® were assessed during the trial. Collected blood samples were analysed through the LC-MS/MS bioanalytical method. CBD was detected at the 30 min and 60 min post-administration for the CBD-pro-Pheroid® and the pure CBD products, respectively. A significantly higher mean CBD C_{max} of 4.45 ± 3.11 ng/mL was reported for CBD-pro-Pheroid® compared to a mean of 0.18 ± 0.23 ng/mL reported for pure CBD. The $AUC_{0-\infty}$ was documented as 17.09 ng/mL/h and 3.28 ng/mL/h for CBD-pro-Pheroid® and pure CBD, respectively. It was evident from these results that the absorption of the CBD-pro-Pheroid® formulation was

significantly higher than the pure CBD, confirming that the pure CBD is ineffective in crossing the biological membranes without an effective vehicle.

The CBD bioavailability of the test formulation was favourable when compared to the reference compound and to current literature. Since pure CBD is rarely administered to patients in general practice, a relative bioavailability could not be reported and the extent to which the bioavailability was increased by using Pheroid® remains unconfirmed. The enhanced bioavailability, together with an advantageous safety profile, provides the rationale for the use of pro-Pheroid® as the drug delivery system for CBD and supports the future development of a CBD-pro-Pheroid® formulation.

Keywords: Cannabidiol; CBD-pro-Pheroid®; Pheroid® technology; Pharmacokinetics; Drug safety; Bioavailability; Drug formulation; Stability testing; Characterisations; Phase 1 clinical trial

OPSOMMING

Die terapeutiese voordele van suiwer Cannabidiol (CBD), 'n fitokannabinoïde geïsoleer vanuit die *Cannabis sativa L*. plant, word ondersteun deur ‘n groeiende hoeveelheid terapeutiese effekte van CBD, waar die middel gebruik word in ‘n wye spektrum van kondisies wat wissel vanaf aanvullend tot die behandeling van ernstige en aftakelende epilepsie. Die terapeutiese effekte, saam met die gebrek aan nadelige effekte, bied die rasionaal vir die kroniese gebruik van CBD. Daar is egter beperkinge in die aflewering en die stabiliteit van die CBD. Die grootste uitdaging van CBD is die lipofiele aard van CBD, wat lei na 'n lae biobeskikbaarheid van 6%. 'n Effektiewe medisyne-afleweringstelsel, Pheroid®, was gebruik om potensieël die eienskappe van CBD te verbeter, met klem op die biobeskikbaarheid. ‘n CBD-pro-Pheroid® formulering was ontwikkel en geoptimaliseer. Die geoptimaliseerde middel (tesame met 'n pro-Pheroid®-kontrole) was onderwerp aan ses maande versnelde stabiliteitstoetsing by 5°C, 25°C/60% RH, 30°C/70% RH en 40°C/75% RH. Morfologiese analise, deeltjiegrootte verspreiding en zeta potensiaalmetings was uitgevoer. Die pH, die integriteit van die kapsule en die CBD konsentrasies was adisioneel ook bepaal om 'n omvattende profiel van die CBD-pro-Pheroid® te verkry. Die Pheroid®-tegnologie het die CBD gedurende ses maande gestabiliseer en verbeterde resultate was vir die geoptimaliseerde formulering gedokumenteer. Die voorlopige studie resultate het vir CBD vir ses maande by 25°C/60% RH die beste stabiliteit getoon. Resultate bevestig die behoefte aan behoorlike formulering en stabiliteitstoetsing vir algemene CBD-produkte.

Die hoof doel van die studie was om die biobeskikbaarheid van die geoptimaliseerde CBD-pro-Pheroid® formulering te evalueer. Dit is bereik deur 'n fase 1 kliniese proef uit te voer, waar die CBD-pro-Pheroid® (wat 20 mg suiwe farmaseutiese graad CBD en 450 mg pro-Pheroid® bevat het) as 'n toetsformulering toegedien is en suiwer CBD as 'n kontrole middel toegedien is. Die studie het 14 gesonde deelnemers (beide manlik en vroulik) betrek in 'n willekeurige oorgangsondersoek, enkel-dosis, enkel-sentrum studie-ontwerp. Die farmakokinetiese waardes en die veiligheidsprofiel vir die geoptimaliseerde formulering was tydens die studie gedokumenteer. Opeenvolgende bloedmonsters is vir 48 uur versamel om 'n omvattende profiel van die formulasies te verkry. CBD was opgespoor in die 30 min bloedmonster vir die CBD-pro-Pheroid® en in die 60 min bloedmonster vir die suiwer CBD. Die absorpsie van die CBD-pro-Pheroid® formulering was aansienlik verbeter relatief tot die suiwer CBD. 'n Gemiddelde CBD C_{max} van 4,45 ± 3,11 ng/mL is tussen 1 en 1,5 uur bereik vir CBD-pro-Pheroid® en 'n gemiddeld van 0,18 ± 0,23 ng/mL is tussen 1,5 en 2 uur vir suiwer CBD verkry. Die $AUC_{0-\infty}$ was onderskeidelik 17,09 ng/mL/h en 3,28 ng/mL/h vir die CBD-pro-Pheroid® en die suiwer CBD. Dit bevestig dat suiwer CBD nie die biologiese membrane kan oorsteek sonder die hulp van ‘n effektiewe vervoermiddel nie. Die CBD-biobeskikbaarheid van die toetsmiddel was gunstig in

vergelyking met die verwysingsformulering en die huidige literatuur. Aangesien CBD egter selde in die praktyk aan pasiënte toegedien word in die suiwer vorm, kon 'n relatiewe biobeskikbaarheid nie bepaal word nie. Die mate waarin die biobeskikbaarheid verhoog is, bly onbevestig. Tesame met die verbeterde biobeskikbaarheid was slegs geringe newe effekte en geen ernstige newe effekte gedokumenteer nie. Dit bied 'n belangrike rede vir die gebruik van pro-Pheroid® vir die toediening van CBD en dit bied belowende resultate vir die toekomstige ontwikkeling van 'n CBD-pro-Pheroid®-formulering.

Sleutelwoorde: Cannabidiol; CBD-pro-Pheroid®; Pheroid®-tegnologie; farmakokinetika; Veiligheid; Biobeskikbaarheid; Formulering; Stabiliteitstoetsing; Karakteriserings; Fase 1 kliniese proef

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LIST OF ABREVIATIONS

AE	Adverse event	**C_{max}**	Maximum concentration
AEA	Anandamide	**CO_2**	Carbon dioxide
AD	Anno Domini	**CoA**	Certificate of analysis
ALP	Alkaline phosphatase	**CV**	Coefficient of variation
ALT	Alanine aminotransferase	**DST**	Department of Science and Technology
ANOVA	Analysis of variance	**ECS**	Endocannabinoid system
API	Active pharmaceutical ingredient	**EDTA**	Ethylenediaminetetraacetic acid
ARV	Anti-retroviral drug	**EMA**	European Medicines Agency
AST	Aspartate aminotransferase	**ESI**	Electron spray ion
AUC	Area under curve	**FDA**	Food and Drug Administration
BC	Before Christ	**GGT**	Gamma-glutamyl transferase
BP	Blood pressure	**GLP**	Good Laboratory Practices
CAS	Chemical abstracts service	**GPCR**	G-protein coupled receptors
CB	Cannabinoid	**GRAS**	Generally Recognised As Safe
CBC	Cannabichromene	**H_2O**	Water
CBD	Cannabidiol	**HCL**	Hydrochloric acid
CBDA	Cannabidiolic acid	**HPLC**	High Performance Liquid Chromatography
CBN	Cannabinol	**HR**	Heart rate
CDER	Center for Drug Evaluation and Research	**HREC**	Health research ethics committee
cGMP	Current Good Manufacturing Practices	**ICF**	Informed Consent Form
CLSM	Confocal Laser Scanning Microscopy	**ICH**	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

IS	Internal standard	**PI**	Principal investigator
IV	Intravenous	**PK**	Pharmacokinetic
LC-MS/MS	Liquid-chromatography-tandem mass spectrometry	**p.o**	*Peroral*
LD_{50}	Median lethal dose	**RA**	Regulatory authority
LLOQ	Lower limit of quantification	**ROS**	Reactive Oxygen Species
Max	Maximum	**SA**	South Africa
MCC	Medicine Control Council	**SAE**	Serious Adverse Event
MF	Matrix factor	**SAGCP**	South African Good Clinical Practices
Min	Minimum	**SAHPRA**	South African Heath Product Regulatory Authority
MRM	Multiple reaction monitoring	**SD**	Standard deviation
MS	Multiple sclerosis	**SEDDS**	Self-emulsifying drug delivery system
N_2O	Nitrogen oxide	***sp***	Species
NaOH	Sodium hydroxide	**THC**	Tetrahydrocannabinol
NWU	North-West University	**UK**	United Kingdom
p	Probability	**US**	United States
DST/NWU PCDDP	Preclinical Drug Development Platform	**Vd**	Volume of distribution
PCI	Principal clinical investigator	**WHO**	World Health Organisation

LIST OF UNITS

®	Registered trademark	**mV**	Millivolt
%	Percentage	**m/Z**	Mass divided by charge
g	Gravitational force	**ng**	Nanogram
h	Hours	**nm**	Nanometer
kg	Kilogram	**RH**	Relative Humidity
kPA	Kilopascal	**µm**	Micrometer
mg	Milligram	**µg**	Microgram
min	Minute	**µL**	Microliter
mL	Milliliter	**v/v**	Volume per volume
mm	Millimeter	**°C**	Degrees Celsius
msec	Millisecond		

Cannabis	Refers to the scientific name for the *Cannabis* plant
cannabis	Refers to the recreational/ medicinal drug

CHAPTER 1: STUDY JUSTIFICATION, AIM AND OBJECTIVES

1.1 BACKGROUND

Cannabis sativa L., originating from China and well documented since 4000 BC., is one of the most versatile plants known today, considering that it is cultivated as a valuable source of nutrients and fabrics. It is also used for ritual, recreational and a wide range of therapeutic purposes (Ben Amar, 2006; Li, 1974; Guy *et al*., 2008). The potential therapeutic benefits of *Cannabis sp.* in chronic conditions such as epilepsy, neuropathic pain and multiple sclerosis (MS), among numerous others, have been demonstrated in recent years (Collin *et al*., 2007; Ellis *et al*., 2009; Szaflarski & Bebin, 2014). Insight into these therapeutic benefits and the pharmacological effects of *Cannabis sp.* was provided with the discovery of 70 distinctive phytocannabinoids isolated from the plant. The two main phytocannabinoids are known as Delta-9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD) (Elsohly & Slade, 2005; Pertwee, 2006).

Pure CBD has gained increasing attention in the past two decades for its potential medicinal properties including anti-psychotic, neuroprotective, anti-emetic, anti-inflammatory, anxiolytic and anti-convulsant effects (Zhornitsky & Potvin, 2012; Zuardi, 2008). The use of CBD is also receiving growing recognition as it has proved to be well-tolerated and void of adverse events (AEs) often associated with the administration of cannabis. The latter includes psychoactive effects, lowered blood pressure, depersonalisation and tachycardia. These effects can be primarily attributed to the use of THC (Hirst *et al*., 1998; Zuardi, 2008). Due to the therapeutic benefits and the favourable safety profile of CBD this investigation focused on pure CBD as the Active Pharmaceutical Ingredient (API). The use of pure CBD is currently a schedule 4 pharmaceutical drug in South Africa when indicated for therapeutic use, based on the Medicines and Related Substances Act 101 of 1965. Importantly, since 15 May 2019, CBD is exempt from scheduling for a period of 12 months when administered at low doses to promote general health, without reference to a specific condition (DoH, 2019).

1.2 PROBLEM STATEMENT

CBD is primarily administered through inhalation, orally as a capsule or dissolved in an oil. It is also administered as a sublingual spray (Guy & Flint, 2004; Millar *et al*., 2019; WHO, 2018). The oral administration of pharmaceutical drugs provides advantages above alternative administration routes. These advantages include patient compliance, a predicTable dosage regime, increased safety and simplicity in manufacturing and usage. It is therefore beneficial to develop an improved CBD formulation for oral administration (Anselmo & Mitragotri, 2014; Cherniakov *et al.*, 2017).

However, the absolute bioavailability of orally administered CBD, in humans, is documented in limited sources in literature. A single study by Ohlsson *et al*. (1986) reported an absolute oral bioavailability of 6%. This low oral bioavailability is problematic and presumably occurs due to the high lipophilic nature and the extensive first pass metabolism of cannabinoids (Grotenhermen, 2003; Zhornitsky & Potvin, 2012). Drug characteristics, such as drug bioavailability, can be modified during drug formulation. Drug delivery systems are particularly beneficial during drug formulation since it can be used to maximise drug therapeutic efficacy, decrease dosage requirements or to improve a compound's oral bioavailability. Drug delivery systems can also reduce the side effects associated with the API, depending on the desired outcome (Wen *et al*., 2015). During this investigation the lipid-based Pheroid® drug delivery system was selected as a particle delivery system to potentially ameliorate the bioavailability challenges of a *peroral* CBD formulation.

Pheroid® technology was of interest as a drug delivery system for the current study since its use has previously been successfully attributed to increasing the bioavailability of pharmaceutical compounds such as hormonal, anti-microbial, anti-malarial and anti-tuberculosis drugs (Grobler, 2009; Grobler, 2014; Nieuwoudt, 2009; Steyn, 2010). In addition, Pheroid® is comprised of compounds with GRAS (Generally Recognised As Safe) status with a good safety profile. However, the main rationale for using Pheroid® instead of alternative drug delivery systems was the recent confirmation that Pheroid® technology successfully increased the oral bioavailability of pure CBD in rats. This was demonstrated in an *in vivo* preclinical study by Cloete (2017), where a novel CBD-Pheroid® formulation was compared to commercially available products. Van Wyk (2018) assessed the effects of a CBD-Pheroid® formulation on the central nervous system and documented no significant behavioral effects after intravenous (IV) administration in conscious rats. The obtained results of both studies supported the potential clinical use of the Pheroid® drug delivery system for an increased oral bioavailability of CBD.

1.3 STUDY JUSTIFICATION

Considering the low bioavailability and the insufficient information on pure CBD in literature, this study was executed to potentially develop an improved *peroral* CBD formulation. An improved *peroral* CBD formulation would be advantageous by potentially increased plasma CBD concentrations. This could result in a corresponding increase in therapeutic benefits, reducing the need for high-cost dosages.

Since limited data is available on the stability testing of CBD preparations, this study contributed to the shortage of documented stability studies on CBD-formulations and provided supplementary information on the Pheroid® drug delivery system. Furthermore, this study has the potential to be the foundation for the future development of a high-quality pharmaceutical grade CBD drug in South Africa. Currently only one pure CBD product (Epidiolex®; developed by GW Pharmaceuticals) has up to date been registered globally for medicinal purposes. The justification for an improved *peroral* CBD formulation, and consequently this study, thus resides in the future potential of this therapeutic wide-spectrum drug.

1.4 AIM AND OBJECTIVES

The primary aim of the study was to evaluate the bioavailability of a *peroral* CBD formulation when encapsulated in the Pheroid® drug delivery system.

To achieve this aim, the following objectives were specified for this study:

1) Formulating a CBD-pro-Pheroid® product, in compliance with current Good Manufacturing Practices (cGMP), and analysing the formulation characteristics by means of confocal imaging, particle size distribution and zeta potential measurements.

2) Evaluating the stability of CBD-pro-Pheroid® formulation by conducting a formal stability study for six months.

3) Conducting an ethically approved phase I clinical trial, in compliance with Good Clinical Practices (GCP), to assess the pharmacokinetic (PK) profiles and the safety associated with the use of the CBD-pro-Pheroid® formulation.

1.5 STUDY OUTLINE

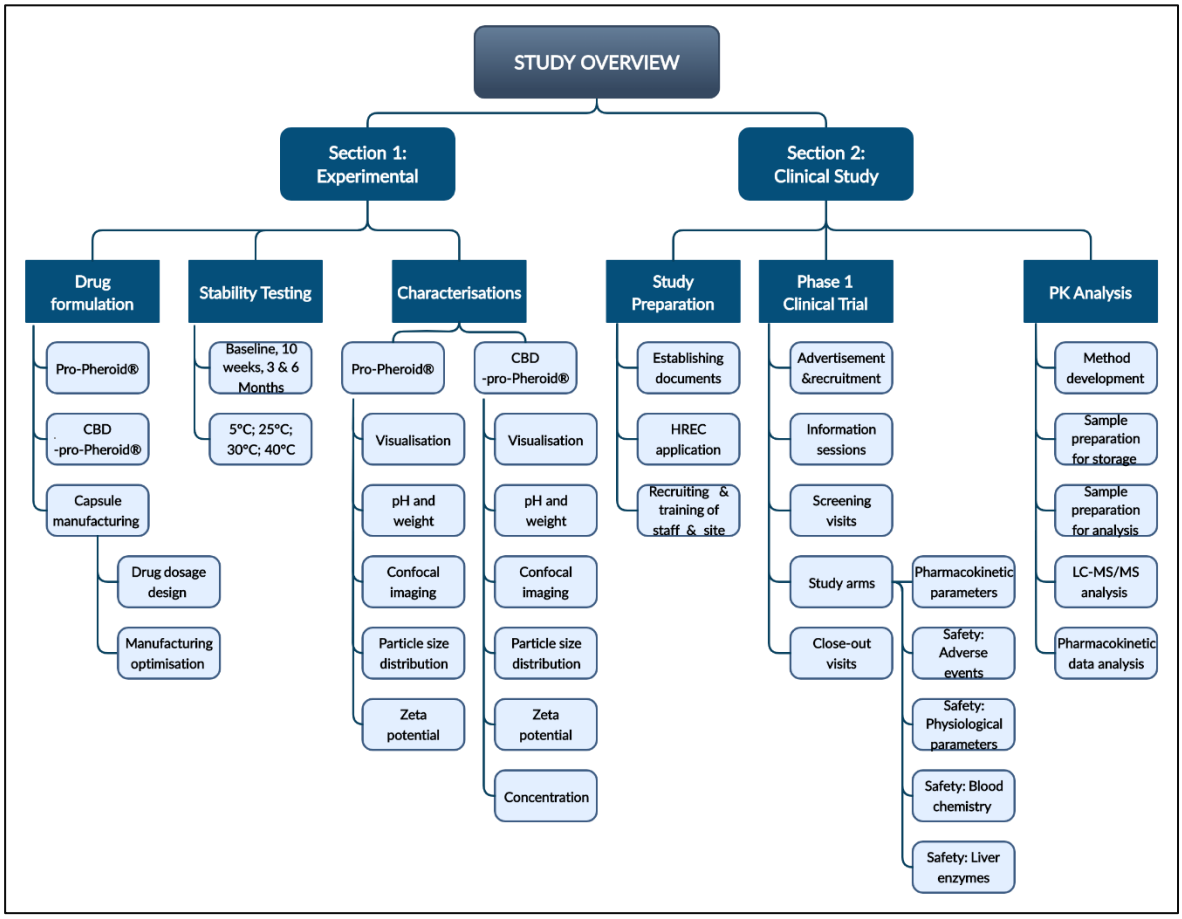


Figure 1-1: A schematic representation of the tasks performed during the respective study phases.

1.6 DISSERTATION OUTLINE

Table 1-1: An outline of the respective study chapters included in the dissertation.

Chapter 1:	A brief introduction to the study including the justification for the study, the scope and objectives of the study.
Chapter 2:	A summary of the existing relevant literature; with the focus on the dosage and PK values obtained from CBD administration in clinical studies. It also highlights the pitfalls of the current literature on CBD.
Chapter 3:	An article assessing the characteristics of CBD-pro-Pheroid® formulations following formal stability testing at specified conditions. This chapter includes

	information on the morphology, the particle size, the zeta potentials and the concentrations of the formulations.
Chapter 4:	A research manuscript for publication that evaluates the administration of the *peroral* CBD-pro-Pheroid® formulation to healthy participants during a phase 1 clinical trial and the effects of the pro-Pheroid® formulation on the PK values of CBD.
Chapter 5:	The current study limitations and future recommendations are addressed. The study conclusion and final statements are also provided.
Annexures:	Included in the annexures are the relevant documents used during the clinical trial, including the certificate of ethical approval for the phase 1 clinical trial (NWU-00020_18_A1) and the detailed results which are summarised in the respective chapters of the dissertation.

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CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Cannabinoids (CBs) are a diverse group of chemical compounds with an affinity for the cannabinoid receptors and the endocannabinoid system (ECS). CBs are characterised into three subgroups namely endocannabinoids (endogenous), synthetic cannabinoids (chemically synthesised) and phytocannabinoids (plant-derived) (Franguas-Sánchez *et al*., 2016). The discovery and subsequent isolation of phytocannabinoids have led to a gradual increase of interest into *Cannabis*-based research until 1975, after which a decline in publications were observed. However, a recent upsurge in attention to *Cannabis* occurred essentially due to the confirmation of an abundance of potential therapeutic effects associated with the use of isolated cannabinoids (Zuardi, 2008).

Cannabis sp. is the primary source of phytocannabinoids and, although the medicinal use of the plant was first documented in the earliest Chinese Pharmacopoeia (100 A.D.), its exact pharmacological effects and potential medicinal uses have still only been partially elucidated (Hanuš *et al*., 2016; Touw, 1981). Phytocannabinoids are found on the flower of the female *Cannabis* plant (figure 2-1); situated in the resin glands. The phytocannabinoids are produced by the glandular trichomes of the plant as a mechanism of defence against herbivores and parasites (Clarke & Watson, 2002; Kumar *et al*., 2019; Widelski & Kukula-Koch, 2017; Zuardi, 2008).



Figure 2-1: Representation of the trichomes, present on *C*. *sativa* leaves, where CBD is expressed (Image provided by S. Erasmus).

Due to conflicting evolutionary interpretations of the genus *Cannabis,* some sources recognise that cultivation has resulted in four species diverged from *Cannabis sativa*. These species are categorised based on their morphology and include *C. sativa*, *C. afghanica*, *C. indica* and *C. ruderalis* (Clarke & Merlin, 2013; Piomelli & Russo, 2016). However, other sources recommend that *Cannabis* must only be classified as *Cannabis sativa* L. (Small, 2015). To promote congruence among literature, this study only refers to *Cannabis sativa* L. Despite the confusion on nomenclature, it is well-understood that there are several *Cannabis* chemotypes. The ratio of chemical compounds had been altered during cultivation to exert different recreational and therapeutic desired effects (Piomelli & Russo, 2016; Russo, 2011).

The *Cannabis* plant contains more than 483 chemical compounds of which more than 66 are phytocannabinoids. Tetrahydrocannabinol (THC), Cannabidiol (CBD), Cannabinol (CBN) and Cannabichromene (CBC) are the phytocannabinoids found in abundant quantities in *Cannabis* (Brenneisen, 2007; Grotenhermen, 2003; Zhornitsky and Potvin, 2012). Although the combined use of cannabinoids has shown to result in a synergistic pharmacological effect, the current study focussed on isolated CBD (Piomelli & Russo, 2016; Russo & Guy, 2006). Pure CBD was of interest due to its wide range of medicinal properties and as it is void of the psychoactive effects normally associated with cannabis use.

2.2 CANNABIDIOL

2.2.1 Chemical properties of Cannabidiol

CBD is a non-psychotropic highly lipophilic phytocannabinoid accounting for up to 40% of the *Cannabis sativa* L. plant (Campos *et al*., 2012). The exact molecular structure of CBD (figure 2-2) was first elucidated in 1963; 23 years after its isolation (Adams *et al*., 1940; Mechoulam & Shvo, 1963). It has now been established that the terpenophenic CBD molecule is produced by the natural decarboxylation of cannabidiolic acid (CBDA) during exposure to light, heat or aging (Mechoulam & Hanuš, 2002; Russo, 2017).

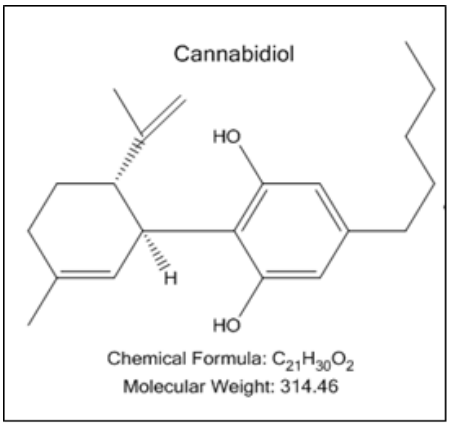


Figure 2-2: Representation of the molecular structure of CBD adapted from Huestis (2007) on ChemDraw Software version 12.0.

The isolation of CBD prompted the investigation into the pharmacological action of pure CBD, with less attention been given to the related therapeutic benefits during the period ranging from 1940 to 1970 (Pertwee, 2006).

2.2.2 Pharmacodynamic properties of Cannabidiol

It is well-known that the phytocannabinoids interact with the ECS, which is described as a complex signalling system consisting of cannabinoid receptors, endogenous ligands and enzymes (Di Marzo *et al*., 2004). CBD has also recently been described by several sources, including Premoli *et al.* (2019) and Morales *et al*. (2017), as a multi-target drug. This is because CBD not only exerts an action on the ECS but also interacts with other G-protein coupled receptors (GPCRs) including opioid and serotonin receptors. Pharmacological research on the cannabinoids has aided in the pivotal discovery of two important G-protein-coupled cannabinoid receptors namely CB_1 and CB_2. CB_1 is primarily localised in the central nervous system and CB_2 can be found in the peripheral nervous system, immune cells, spleen and tonsils. Unlike THC, CBD exhibits a low affinity for and does not activate or block either CB_1 or CB_2 (Fine & Rosenfield, 2013; Pertwee, 2006; Svíženská *et al*., 2008). It was proposed that the anti-inflammatory properties of CBD are produced by the agonist activity of CBD at CB_2 receptors. CBD is considered as a negative allosteric modulator of both CB receptors and has the ability to inhibit cellular uptake of the endogenous CB_1 ligand, anandamide (AEA) (Fine & Rosenfield, 2013; Morales *et al*., 2017). Furthermore, CBD is evidenced to play a major role in inhibiting certain cytochrome P450 enzymes (mainly the 2C and 3A enzymes). When administered concomitantly with THC the described inhibition results in a decrease in the conversion of THC to 11-OH-THC and ultimately in a reduction of THC-induced psychotic symptoms. This provides a rationale for the synergistic use of CBD and THC (Englund *et al*, 2013; Karschner *et al*., 2011; Zhornitsky & Povin, 2012).

Although these studies have provided some insight into the pharmacological action of CBD, the exact mechanism of action and signalling routes remain unclear, as it is unknown whether CBD completely binds to a specific receptor site (Mecha *et al*., 2017). Investigational research and studies are ongoing to fully explicate the molecular pharmacology of CBD in order to fully comprehend the therapeutic effects attributed to CBD use (Morales *et al*., 2017).

2.2.3 Therapeutic indications of Cannabidiol

The number of clinical trials investigating the therapeutic effects of CBD has increased in recent years. CBD is confirmed to be clinically relevant due to its anti-psychotic, anti-emetic, anti-inflammatory, anxiolytic and anti-convulsant properties (Pertwee, 2005; Rudroff & Honce, 2017; Zhornitsky & Potvin, 2012). Presented in Table 2-1 are the results obtained from clinical studies investigating a number of these properties.

Table 2-1: Potential therapeutic uses of CBD reported in clinical studies.

Clinical Studies					
Medical/ health condition		**Study type n =Number of subjects**	**(D): Dose (R): Route of administration**	**CBD therapeutic benefit**	**Reference**
1	Anxiety, social anxiety disorder	Double-blind placebo-controlled randomised trial n = 24	(D) = 600 mg acute dose (R) = p.o	Significant reduction in anxiety, cognitive impairment and discomfort	Bergamaschi *et al.* (2011)
2	Dravet Syndrome/ Epilepsy	Double-blind, placebo-controlled trial n = 120	(D) = 20 mg/kg for 14 weeks (R) = p.o	Significant reduction in frequency of convulsive seizures	Devinsky *et al*. (2017)
3	Multiple sclerosis	Double-blind, randomised placebo-controlled cross-over trial n = 18	(D) = 2.5 – 200 mg/day for 2 weeks (R) = Sublingual spray	Significant reduction in pain	Wade *et al.* (2004)
4	Schizophrenia/ Psychosis	Double-blind placebo-controlled parallel-group trial n = 88	(D) = 1000 mg/day for 8 weeks (R) = p.o	Lower levels of psychotic symptoms and improved wellbeing	McGuire *et al. (*2017)
Abbreviations: p.o = *Peroral*					

Additional promising therapeutic effects in several other conditions have been confirmed by preclinical animal study results, but currently lacks confirmation in well-designed and executed clinical trial results (Iffland & Grotenhermen, 2017; Zuardi, 2008). Presented in Table 2-2 are the results obtained from preclinical studies investing the therapeutic benefits of CBD.

Table 2-2: Potential therapeutic uses of CBD reported in preclinical studies.

i Preclinical *in vivo* studies					
Medical/ health condition		**Subject description n = Subject count BW = Body weight**	**(D): Dose (R): Route of administration**	**CBD therapeutic benefit**	**Reference**
1	Addiction treatment	Male C57BL/6 mice - Drug conditioned n = 100 BW = 25 – 30 g	(D) = 2.5, 5, 10, and 20 mg/kg/mL acute dose iii (R) = i.p	CBD blocked opioid reward, Drug-place preference was attenuated	Markos *et al.* (2018)
2	Alzheimer's disease/ dementia/ memory loss	3–5-month old C57BL/6J mice n = NR BW = 35 – 40 g	(D) = 10 mg/kg for 7 days (R) = i.p	Anti-inflammatory properties: Significant attenuation of the reactive gliosis induced by β-amyloid peptide injury	Esposito *et al*. (2007)
3	Depression	Male Swiss mice n = 6 – 10 per group BW = 20 – 25 g	(D) = 3, 10, 30, 100 mg/kg acute dose (R) = i.p	CBD induces antidepressant-like effect	Zanelati *et al.* (2010)
4	Diabetes mellitus Type 1	6–12-week old female NOD mice n = 20 BW = NR	(D) = 5 mg/kg/day 5 times a week (R) = i.p	Significant reduction in incidence of diabetes development & the plasma levels of pro-inflammatory cytokines	Weiss *et al*. (2006)
5	Lung cancer	Athymic nude n =4 per group BW = NR	(D) = 5 mg/kg/d for 7 days (R) = Subcutaneous	Inhibited cancer cell invasion and metastasis.	Ramer *et al.* (2012)
6	Neonatal ischaemia	New-born piglets n = 29 BW = 1.7 ± 0.1 kg	(D) = 0.1 mg/kg acute dose (R) = IV	Histological, functional, biochemical & neurobehavioral improvements	Lafuente *et al.* (2011)
7	Neuropathic pain	Male Wistar rats n = NR BW = 200–220 g	(D) = 2.5, 5, 10 or 20 mg/kg for 1 week (R) = p.o	Successful reversal of both thermal & mechanical hyperalgesia	Costa *et al.* (2007)
Abbreviations: i.p = Intraperitoneal, IV = Intravenous, NOD = Non-obese diabetic, NR = Not reported, p.o = *Peroral*					

As observed from literature, a plethora of therapeutic effects are attributed to the use of CBD. To maximise the therapeutic effects of CBD it is essential to assess how CBD is circulated within the body, from administration to excretion (Huestis, 2007). An overview of the current Pharmacokinetic (PK) properties of *peroral* CBD is provided in section 2.2.4.

2.2.4 Pharmacokinetic properties of *peroral* Cannabidiol

Phase 1 clinical trials are a pivotal part in the drug development process where, following preclinical confirmation, information regarding the PK values and the safety associated with the drug use is reported (Akhondzadeh, 2016; Bergström & Långström, 2005). PK describes the drug concentration-time course through the body and includes aspects such as drug absorption, distribution, metabolism and excretion to better understand the movement of the drug through the biological system (Aulton, 2002).

Administration: Multiple routes of CBD administration have previously been investigated in clinical trials, depending on the desired therapeutic outcome. These include topical, inhalation, sublingual, buccal and, to a lesser extent, parental administration (Chelliah *et al*., 2018; Cherniakov *et al*., 2017; Guy & Flint, 2004; Millar *et al*., 2019; Ohlsson *et al*., 1986). However, the prevalent method of administration is the oral route as either a capsule or an oil (WHO, 2018).

Oral administration is also the preferred method of administration in clinical trials, since accurate dosages can be administered, patient compliance is improved and it is easy to administer (Anselmo & Mitragotri, 2014; Cherniakov *et al*., 2017; Dressman & Reppas, 2000).

With the chronic use of CBD, capsules can potentially provide additional advantages above CBD oil. As CBD is sensitive to both oxygen and light, continued exposure (as typically found with the use of an oil) can affect the drug stability (Trofin *et al*., 2012). Capsules can provide protection against environmental conditions including light, oxygen and contaminants. Furthermore, other CBD administration forms (such as Sativex®) reportedly have an inherent bitter taste which usually needs to be masked during clinical trials (Lus *et al*., 2018; Steup, 2018). The use of capsules can also ameliorate this unwanted trait of CBD products. The current study focussed on the administration of pure CBD in gelatin liquid capsule form.

The doses administered in clinical trials vary to a great extent. The oral CBD dosages used in diseased states range from <1 to 50 mg/kg/day. Details regarding the doses are summarised in a review by Millar *et al.* (2019). CBD is currently, until May 2020, provisionally exempt in South Africa from scheduling for dosages not exceeding 20 mg/day and administered for general health only (DoH, 2019). There is a lack of literature regarding information on recommended CBD doses when indicated for daily use to promote general health.

Absorption: The absorption of CBD is slow, erratic and variable, resulting in high inter-subject variability (Eichler *et al*., 2012; Grotenhermen, 2003). Absorption can be influenced by multiple factors, such as the formulation of the drug, genetics, food interactions and adiposity of the subject (Grotenhermen, 2003; Huestis, 1999; Millar *et al*., 2018). The onset of action is delayed after CBD administration, with the C_{max} (the maximal drug serum concentration) documented as ranging between 30 – 120 min after oral administration (Guy & Robson, 2004; Nadulski *et al*., 2005b). The C_{max} and AUC are reported to be dose-dependent. In contrast, the T_{max} does not follow the same trend as it is independent of the administered dose. The half-life ($T_{1/2}$), defined as the period required for the concentration of the drug in the body to be reduced by one-half, was found to be longer in fasting (38.9 h) subjects compared to fed (24.3 h) subjects after a single oral dose of 200 -300 mg CBD was administered (Birnbaum *et al*., 2019; Shargel *et al*., 2005). Summarised in Table 2-3 are the AUC and C_{max} values obtained during studies investigating the PK properties of acute, low dose (< 50 mg) *peroral* CBD formulations. PK values are substantially variable in literature. It is noteworthy that the vehicle of the CBD administration is either not reported in detail or not similar to other studies. Importantly, the majority of PK studies, presented in Table 2-3, reported males rather than both sexes as study subjects. An emphasis is placed on

including females in future CBD studies for comprehensive information of the CBD PK parameters.

Table 2-3: Summarised mean PK data of acute low dose CBD administration.

No	Dosage form	Vehicle	CBD dose (mg)	Sex	AUC (ng/mL.h)	Cmax (ng/mL)	Tmax (h)	Reference
1	Oral	Granulated lactose	10	Male & female	5.76	2.47	1.27	Guy & Robson (2004)
2	Sublingual	Elycerol: Ethanol: Propylene Glycol	20	Male & female	2.60	2.05	2.17	Guy & Flint (2004)
3	Oral	NR	5.4	Male & female	4.35	0.93	1.00	Nadulski *et al*. (2005a)
4	Oral	Ethanol	14.8	Male	7.67	3.95	1.17	Eichler *et al*. (2012)
5	Oromucosal spray	NR	40	Male	3.23	1.03	1.00	Stott *et al.* (2013)
6	Buccal	Piperine-PNL	10	Male	6.90	2.10	1.00	Cherniakov *et al*. (2017)
7	Oral	NR	10	Male	8.89	2.97	2.97	Atsmon *et al.* (2018)
Abbreviations: ND = Not reported								

Distribution: CBD has a high volume of distribution (Vd) of approximately 32 L/kg. As previously discussed, CBD is known for its high lipophilic characteristic, with a reported Log*P* of 6.50 (Pubchem, 2019). Piccaro *et al.* (2015) described that the Log*P* value of a drug is useful to indicate drug affinity properties. Drugs with a Log*P* of 0 are hydrophobic and a LogP between 0 – 3 indicates a moderate lipophilic nature, which is optimal for drug formulation. In contrast, compounds with a Log*P* > 5, such as CBD, tend to accumulate in biological tissues containing lipids. Accordingly, CBD readily distributes to tissues such as the brain and adipose tissue (Grotenhermen, 2003; Ohlsson *et al*., 1984).

Metabolism: The CBD bioavailability, defined as the proportion of the pharmaceutical drug which enters the circulation, has recently been the focal point of a number of clinical studies. CBD is subjected to substantial first-pass metabolism in the gastrointestinal tract. This, together with the confirmed highly lipophilic nature of CBD, results in a reported oral bioavailability of 6% (Grotenhermen, 2003; Millar *et al*., 2018; Ohlsson *et al*., 1986; Zhornitsky & Potvin, 2012). This is low when compared to alternative administration routes, such as inhalation, with a reported systemic bioavailability of 31% (Grotenhermen, 2003; Ohlsson *et al*., 1986). The low bioavailability of CBD can potentially restrict the wide range of therapeutic benefits as well as the efficacy of CBD in related conditions.

Liver P450 enzymes (CYP3A (2/4) and CYP2C (8/9/19) enzymes) are predominantly responsible for the hydroxylation of CBD to 7-OH-CBD and other derivatives. These metabolites are then subjected to further liver metabolism, where after they are oxidised resulting in the formation of CBD-7-oic acid. Glucuronides of these metabolites can also be formed (Grotenhermen, 2003; Harvey & Mechoulam, 1990; Mechoulam & Hanuš, 2002). In a clinical trial investigating CBD metabolites, it was found that the prevalent circulating metabolite detected in the plasma was 7-carboxy-CBD, followed by the parent active molecules 7-OH-CBD and 6-OH-CBD (Taylor *et al*., 2018).

Excretion: The metabolites are either excreted intact or as glucuronide conjugates in the faeces and urine within 72 h after a single dose administration (Devinsky *et al*., 2014; Ujváry & Hanuš, 2016). CBD plasma clearance ranges from 960 to 1560 mL/min (Grotenhermen, 2003; Ohlsson *et al*., 1984).

2.2.5 Safety and tolerability

With the discovery of the wide spectrum of CBD therapeutic effects and considering the well-known negative side effects associated with the use of cannabis, it was crucial to establish the safety of CBD when administered without THC. Cannabis was reported to be the most widely used illicit drug world-wide, with 24 million persons over the age of 12 using the drug often and an increasingly high rate of reported dependence (SAMHSA, 2017). Recent studies have found that CBD use, unlike cannabis use, presents no indication for dependence and in contrast is potentially effective in attenuating drug withdrawal symptoms (Hindocha *et al*., 2018; Hurd *et al*., 2015). CBD has indeed been investigated in preclinical studies for therapeutic effects in opioid, cocaine, psychostimulant, cannabis and tobacco addiction. However, there are limited studies investigating this and insufficient data is available (Prud'homme & Jutras-Aswad, 2015).

The safety of CBD, isolated from *C.sativa,* is well-documented and CBD presents a good safety profile. In general, a low toxicity is reported with CBD, but the median oral lethal dose (LD_{50}) has not yet been established in humans (Rosenkrantz *et al*., 1981; Scuderi *et al*., 2009). An IV LD_{50} of 212 mg/kg of CBD was reported in Rhesus monkeys. An exceptionally high oral dose ranging between 20 and 50 times larger than the IV LD_{50} dose was found to result in intoxication in the monkeys (Rosenkrantz *et al*., 1981). Additionally, high oral doses of up to 1500 mg/day have been used in clinical trials and were well-tolerated by patients without causing psychoactive effects usually attributed to cannabis use. (Bergamaschi *et al*., 2011; Grotenhermen *et al*., 2017). CBD is well-tolerated in healthy volunteers and patients, with no or minor AEs reported in several CBD-administered clinical trials (McGuire *et al*., 2017). Physiological parameters including heart rate, blood pressure and body temperature are also not altered by CBD use (Bergamaschi *et al*.,

2011). AEs previously reported with the use of CBD include diarrhoea, tiredness and changes in appetite and in weight (Iffland & Grotenhermen, 2017).

AEs associated with the use of *peroral* CBD reported in a few recent (the past 15 years) clinical trials are categorised in Table 2-4. It is evident that both chronic and acute CBD administration is safe, even at high dosages, with no significant AEs reported during the administration of either acute or chronic pure CBD dosages. The incidence of AEs can potentially increase with chronic concomitant use of medication. This has been documented mostly when the studies were investigating the benefits of CBD in the treatment of epilepsy. A clear interaction between commonly used epilepsy medication and CBD have been confirmed by Gaston *et al.* (2017). Devinsky *et al*. (2017) investigated the use of CBD co-administered with standard anti-epileptic medication in adolescent patients with Dravet syndrome (see Table 2-4). Results included an increase in the health benefits attributed to the addition of CBD to the treatment regime, together with an increase in AE incidences when CBD was administered, compared to the placebo group. AEs occurred in 93% of the CBD group patients and in 75% of the placebo group patients. Somnolence (36% of CBD patients vs. 10% of placebo patients), diarrhoea (31% of CBD patients vs. 10% of placebo patients), loss of appetite (28% of CBD patients vs. 5% of placebo patients), and vomiting (15% of CBD patients vs. 5% of placebo patients), were among the reported AEs when CBD was administered concomitantly with anti-epileptic medication. Studies reported in Table 2-4 also additionally confirmed that patients experiencing AEs were mostly using valproate concomitantly (Devinsky *et al.,* 2016; Thiele *et al.*, 2018). This potentially indicates that CBD use is safe when not administered in the presence of medication that interacts with CBD.

Table 2-4: Summarised data regarding adverse events of acute and chronic CBD administration.

Study design		Subjects	Administration route	Dose	AEs related to test group when compared to the control group	Reference
CBD administered alone						
Acute	Double-blind, placebo controlled, cross-over	10 healthy male subjects	Oral (capsules with corn oil)	400 mg	No significant effects on psychological measurements or AEs.	Crippa *et al.* (2004)
	Randomised, placebo controlled, double-blind, crossover	15 healthy male subjects	Oral (capsules)	600 mg	No significant effects on HR, BP, task performance or psychological measurements.	Fusar-Poli *et al.* (2009)
	Double-blind study design placebo controlled	12 male and 12 female subjects (12 healthy subjects, 12 patients with SAD[1])	Oral (capsules with corn oil)	600 mg	No significant effects on HR, BP, skin conductance or psychological measurements.	Bergamaschi *et al*. (2011)
	Randomised, placebo controlled, double-blind, crossover	10 male patients with SAD[1]	Oral (capsules with corn oil)	400 mg	No significant effects on psychological measurements.	Crippa *et al*. (2011)
	Placebo-controlled, double blind, crossover	18 male and 10 female patients with schizophrenia	Oral (capsules)	300 & 600 mg	No significant AEs.	Hallak *et al.* (2010)
	Randomised, placebo controlled, double-blind, crossover	16 healthy male subjects	Oral (capsules)	600 mg	No significant differences between CBD and placebo symptomatic psychological parameters.	Martin-Santos *et al.* (2012)
	Randomised, placebo controlled, double-blind, crossover	30 male and female subjects dependent on tobacco	Oral (capsules)	800 mg	No significant psychoactive or AEs.	Hindocha *et al.* (2018)
	Randomised, placebo controlled, double-blind, crossover	12 healthy male and 14 female subjects	Oral (capsules with corn oil)	300 mg	No significant effects on cognitive or psychomotor functions. CBD did not interfere with the sleep cycle of healthy volunteers.	Linares *et al.* (2018)
Chronic	Open label interventional	3 male patients with treatment resistant schizophrenia	Oral	40 -1280 mg/day (35 days)	No AEs.	Zuardi *et al.* (2006)
	Double-blind controlled	42 male and female patients with acute schizophrenia	NR	800 mg/day (2 and 4 weeks)	Fewer side effects were reported with CBD compared to amisulpride.	Leweke *et al.* (2007)
	Active-controlled, double-blind, randomised, parallel-group	32 male and 7 female subjects (37 patients suffering from acute paranoid schizophrenia)	NR	200 mg/day – 800 mg/day (4 weeks)	No significant effect on hepatic or cardiac functions. CBD use showed significantly fewer extrapyramidal symptoms, weight gain and sexual dysfunction compared with amisulpride.	Leweke *et al*. (2012)
	Randomised, placebo controlled, double-blind, parallel group, pilot	62 patients with noninsulin-treated type 2 diabetes (13 treated with CBD) – Male and Female	NR	100 mg twice daily (13 weeks)	Most common AE was reduced appetite. No significant effects on cardiac function, blood count, liver and renal biochemistry.	Jadoon *et al*. (2016)

Co-administered CBD						
Acute	Double-blind, placebo controlled	10 healthy male subjects	Oral	600 mg concomitantly administered with Ketamine	No significant interactive effects of CBD and ketamine on BP, HR, positive symptoms or negative symptoms when compared to placebo group.	Hallak *et al.* (2011)
	Double-blind, placebo-controlled cross-over	9 healthy male and 8 female subjects	Oral (capsules in corn oil)	400 or 800 mg CBD followed by IV fentanyl	The AEs were not aggravated by the addition of CBD.	Manini *et al*. (2015)
Chronic	randomised, double-blind, placebo-controlled	88 male and 83 female patients with treatment resistant Lennox-Gastaut syndrome	Oral (solution)	20 mg/kg daily- in addition to existing medications (14 weeks)	Diarrhoea, somnolence, pyrexia, decreased appetite, and vomiting.	Thiele *et al.* (2018)
	Open-label interventional	2 female patients with BAD[3]	Oral	600 - 1200 mg/day (25 days) with olanzapine	No AEs were observed.	Zuardi *et al.* (2010)
	Double-blind, randomised, placebo controlled	15 male and 6 female patients with PD[2]	Oral (in corn oil)	75 mg/day or 300 mg/day (6 weeks) with standard treatment	No significant AEs effects were observed.	Chagas *et al*. (2014)
	Open-label interventional	147 male and 152 female patients with treatment-resistant epilepsy (162 were in a safety-analysis group – adverse events reported here)	Oral (oil)	2–5 mg/kg/day - 25 or 50 mg/kg/day (12 weeks) with a median of 3 concomitant antiepileptic treatments	AEs reported were decreased appetite, diarrhoea, fatigue, convulsions, appetite changes, lethargy, gait disturbance and sedation. 7% of patients had elevated liver function tests. All patients with hepatic changes used valproate. Diarrhoea or related AEs are reported more with high dose administration >15 mg/kg/day compared to lower doses.	Devinsky *et al.* (2016)
	Open-label interventional	7 male and 6 female child patienst with refractory epilepsy	NR	5 – 25 mg/kg/day (8 weeks) together with CLB[4]	Drug interaction was seen with CLB, where AEs (in 77% of patients) reported were alleviated by reducing CLB dose. CBD was safe and well tolerated.	Geffrey *et al*. (2015)
	Randomised, double-blind, placebo-controlled, parallel group, pilot	60 patients with IBD[5] (Sex: NR)	Oral	50 mg BDS[6] (containing CBD and 4% THC) (2 weeks)	Majority of the reported AEs were mild to moderate and were most likely attributed to the THC.	Irving *et al.* (2015)
	Open-label interventional	4 patients with PD[2] (Sex: NR)	Oral (with corn oil)	150 - 400 mg/day (4 weeks) with standard treatment	No AEs were observed.	Zuardi *et al.* (2009)
	Double-blind, randomised placebo-controlled	62 male and 58 female children and young adult patients with drug-resistant seizures	Oral (solution)	20 mg/kg/day (14 weeks), with standard anti-epileptic treatment	Common AEs were vomiting, fatigue, pyrexia, upper respiratory tract infection, decreased appetite, convulsion, lethargy, somnolence & diarrhoea. More AEs were observed in the CBD group than the placebo group.	Devinsky *et al.* (2017)

[1]SAD- Social Anxiety Disorder, [2]PD-Parkinson's disease, [3]BAD – Bipolar Affective Disorder, [4]CLB – clobazam, [5]IBD – Inflammatory Bowel Disease, [6]BDS - Botanical Drug Substance
[7]NR – Not Reported

In addition to drug dose and concomitant medication (summarised in Table 2-4), two factors that can greatly influence the patient safety are drug formulation and stability (Bajaj, 2012). The quality and stability of the drug are normally assessed during the dosage formulation phases of drug development, prior to registration at a regulatory authority (RA) such as United States (US) Food and Drug Administration (FDA) or the South African Health Products Regulatory Authority (SAHPRA).

2.2.6 Challenges with Cannabidiol: Legal status

The complexity of the legal status of CBD is emphasised as it is still highly controversial in most countries. In the United Kingdom (UK), Germany and several states of the United States of America (USA) CBD has technically been recognised as a new drug, requiring strict safety, quality and effectiveness standards prior to distribution (Hazenkamp, 2018). The conflicting debate is ongoing as some countries, including European Union (EU) countries and South Africa (SA), classify pure CBD as a drug separate from THC with its own safety profile. Other countries, including several states in the USA, on the other hand do not distinguish between CBD and cannabis thus scheduling and categorising all the cannabinoid drugs together as illegal (Mead, 2017). Hazenkamp (2018) reviewed the legal status of CBD and noted that even in countries where CBD use is illegal, products are readily available due to negligence to enforce the law.

There is currently only one pure CBD product (Epidiolex®; GW Pharmaceuticals) worldwide that has been registered by the FDA and marketed for medicinal use. The drug is indicated for severe and often treatment resistant forms of epilepsy. Epidiolex®, but not CBD, is registered as a schedule 5 drug. Schedule 5 indicates the lowest potential for abuse or addiction in the US (Mead, 2017). Despite the proof of pure CBD safety, CBD is still mostly scheduled together with THC. This generalised scheduling has created challenges in CBD research in the past, since THC use is illegal in several countries and associated with safety concerns (Shen, 2014). A full extensive examination of CBD, including abuse potential and associated risks, executed in accordance to FDA regulatory guidance is generally required to altogether reschedule a drug, such as CBD independent from THC.

In addition to Epidiolex®, there are few pure CBD drugs under development and undergoing clinical trials, including Arvisol® (WHO, 2018; Wise, 2018). Other than the products undergoing clinical research, there is a growing magnitude of artisanal CBD-oils in distribution, owing to the interest of CBD as a dietary supplement. This renders the CBD products without the guarantee of product quality or confirmed drug composition (Pavlovic *et al*., 2018). CBD is unregulated in several countries including the EU countries and SA, where the favourable safety profile and the wide spectrum of therapeutic effects associated with pure CBD use resulted in the relaxation of regulation. The use and distribution of pure CBD products in SA, to promote general health, is exempted from scheduling at low dosages (≤ 20 mg) (DoH, 2019; Pavlovic *et al*., 2018).

However, this provisional exemption is valid until 15 May 2020, where high dosage CBD indicated to treat a condition is still classified as a schedule 4 medicine; a drug without abuse potential, by SAHPRA. This is based on the Medicines and Related Substances Act 101 of 1965 (DoH, 2019). This descheduling is highly advantageous for CBD research, while also presenting the risk that CBD-based products are produced and distributed without mandatory analytical testing to determine drug origin, quality, safety, daily dosage, packaging, shelf-life and product stability (Pavlovic *et al*., 2018). The most severe risk this poses is that cannabis obtained from uncontrolled sources is often contaminated with hazardous substances, which may lead to severe health issues. A generalised analytical guideline to guarantee safety, stability and quality of a CBD product is thus essential (Hazenkamp, 2018), even when the outcome is not to register the drug at a RA.

2.2.7 Challenges with Cannabidiol: Drug formulation and stability testing

Apart from the legal challenges and the lack of quality testing, challenges exist in the formulation of a CBD drug. These challenges mainly occur due to the high lipophilic and water insolubility of CBD. The water insolubility of drugs often leads to high inter-and intra-subject variabilities, which have also been observed with the use of CBD (Palmer, 2003). In addition, the heat-, light- and oxygen sensitive characteristics of CBD also present major challenges in drug formulation and drug stability (Gursoy & Benita, 2004; Guy *et al*., 2017; Mechoulam & Hanuš, 2002).

Little is known about the comprehensive stability of CBD-preparations, except for studies investigating the basic properties of CBD. In a study by Trofin *et al.* (2012), the long-term stability of CBD revealed a loss of concentration of 11.08% (4°C in darkness) and 13.44% (22°C in light) from the initial CBD sample after the first year and a loss of CBD concentration of 40% (4°C in darkness) and 42.12% (22°C in light) after 4 years, confirming both the potential sensitivity to light and heat. 14 Commercially available CBD-oils were assessed for quality and CBD oxidation in a study by Pavlovic *et al.* (2018). The study confirmed that evaluating lipid oxidation could provide knowledge on the stability of CBD products. The study also highlighted the difference in cannabinoid profiles and thus the need for strict and standardised regulations, including stability testing and the measurement of CBD concentration within the products.

To potentially address the formulation challenges discussed in this section, a focal point of this study was to introduce a drug delivery system, Pheroid®, to the formulation of CBD.

2.3 PHEROID® DRUG DELIVERY SYSTEM

Therapeutic efficacy, the general rationale for the use of pharmaceutical drugs, can be influenced by poor drug delivery since an adequate amount of the API is required to be delivered to the therapeutic site of action. The use of a drug delivery system can be beneficial as it may facilitate

API delivery. Its use has the potential to result in decreased dosage requirements, improved absorption, improved oral bioavailability and consequently enhanced drug therapeutic efficacy (Gibhard, 2012; Vonarbourg *et al*., 2006; Wen *et al*., 2015). Specific drug delivery approaches to improve oral administration of low aqueous solubility drugs often include micro-emulsions, nanosuspensions and liposomes (Wen *et al*., 2015). During this investigation Pheroid® was selected as the drug delivery system for the clinical trial, due to the promising advantages observed with the use of Pheroid® discussed in section 2.3.

2.3.1 Pheroid® technology overview

The Pheroid® technology forms the basis for a colloidal drug delivery system, attributed to the successful delivery of hydrophobic, hydrophilic and amphiphilic compounds by means of long-chain fatty acids-based nano- and micro vesicles. Following the entrapment in Pheroid® vesicles, the pharmaceutical or biological compounds are delivered across various biological membranes via nasal, oral or transdermal administration routes. The Pheroid® delivery system can be modified regarding the morphology, size, structure and administration route, based on the desired therapeutic outcome (Du Plessis *et al*., 2010; Du Plessis *et al*., 2012; Grobler *et al*., 2008; Grobler, 2009). Furthermore, different Pheroid®-type structures form due to the self-emulsifying properties of Pheroid®, entrapping the API in the Pheroid®. These structures include vesicles (ranging between 0.5 – 1.5 µm), microsponges (ranging between 1.5 – 5 µm) and depots or reservoirs containing pro- Pheroid® (Grobler, 2013). The multiple applications and different structures emphasise the versatility of the Pheroid® as a drug delivery system.

In addition, Pheroid® is stable, inexpensive, easy to manufacture and comprise compounds which are Generally Recognised As Safe (GRAS). This is essential as drug delivery systems must be safe and effective. The Pheroid® consists of an aqueous (H_2O) -, an oil - (primarily of plant and essential fatty acids) and a gas (nitrous oxide; NO_2) phase (Grobler, 2009). For oral administration, a precursor formulation of Pheroid®, known as pro-Pheroid®, was developed. The pro-Pheroid® contains both the oil and gas phases, however, it lacks the aqueous phase. Following administration, the pro-Pheroid® is spontaneously converted *in situ* to Pheroid® vesicles or sponges. Entrapment in the Pheroid® can potentially protect the API from instability, enzymatic degradation or hydrolysis by self-emulsifying characteristics (Grobler, 2009; Grobler, 2013). A self-emulsifying drug delivery system (SEDDS) can be difficult to formulate. However, it is a compelling and vital approach to improve the oral absorption of highly lipophilic drug compounds. SEDDS forms relatively stable oil-in-water (o/w) emulsions and provides advantages above other delivery methods (Gursoy & Benita, 2004; Kohli *et al.,* 2010).

2.3.2 Pheroid® therapeutic applications

The use of the versatile Pheroid® has been shown to result in multiple advantages when investigated in a wide spectrum of preclinical applications. An enhanced absorption and efficacy profile of pharmaceutical and biological compounds for numerous potential applications were observed, including antibiotics, vaccines, agricultural remedies and cosmeceuticals. An increase in bioavailability of a variety of compounds including lumefantrine, ethambutol and artemisone and a decrease in the incidence of drug-related side-effects for pharmaceutical drugs, such as tuberculosis formulations containing rifampicin, pyrazinamide, isoniazid and ethambutol were also documented (Du Plessis *et al*., 2015; Grobler, 2009; Grobler, 2013; Grobler, 2014; Nieuwoudt, 2009). The enhanced efficacy of an API allows for a decrease in the required dosage or a less stringent dosing frequency and may improve patient compliance (Grobler, 2009). The application of Pheroid® in preclinical studies to lumefantrine and rifampicin, is of considerable interest, and shows promise for the current study. Both of these lipophilic drugs were successfully entrapped in the pro-Pheroid® resulting in improved drug bioavailability (Du Plessis *et al*., 2015; Nieuwoudt, 2009).

The North-West University, South Africa, owns some of the intellectual property related to Pheroid® technology. Specific pharmaceutical applications, involving the entrapment of compounds in the Pheroid®, are patented (eight base patents were granted by 2014) (Anon., 2014; Grobler, 2009). Importantly, within the DST/NWU Preclinical Drug Development Platform (PCDDP) group, a comparative preclinical study (in rats) by Cloete (2017) investigated the oral bioavailability of a novel CBD-pro-Pheroid® formulation against four CBD-oils, either commercially available or in development. A single dose of 20 mg/kg resulted in an improved bioavailability compared to the commercially available formulations, with a reported $AUC_{0\text{-}24}$ of 1093.32 ± 470.37 ng/mL. With the improved bioavailability, a decrease was observed in $T_{1/2}$ (6.26 ± 2.87 ng/mL). T_{max} was not influenced by the addition of pro-Pheroid® to CBD. These results provided a promising rationale for the clinical use of Pheroid® for the entrapment of the lipophilic CBD phytocannabinoid.

2.3.3 Pheroid® safety and toxicity

An additional two preclinical studies in rats provided essential support for the clinical study. Firstly, a study by Kleynhans *et al.* (2019) evaluated the sub-chronic toxicity of an upper-limit oral pro-Pheroid® dose. It was found that dosages of 50 mg/kg administered for 90 days were tolerated without any incidence, confirming a baseline safety for pro-Pheroid®. Secondly, Van Wyk (2018) reported no cardiovascular and central nervous effects when administering CBD-pro-Pheroid® formulation at an oral 10 mg/kg dosage.

2.3.4 Pheroid® stability

Due to the instability of CBD, it is important that the potential drug carrier possess the ability to both be stable in biological systems and to render the lipophilic CBD compound stable. Electro-chemical interactions are responsible for sterically stabilising the Pheroid®. This provides Pheroid® with a highly elastic vesicular structure with the ability to cross densely packed capillary walls improving transportation. In addition, accelerated and formal stability testing provided information that the N_2O phase significantly contributes to Pheroid® stability during formulation (Grobler, 2009; Grobler *et al*., 2008).

Attention was given to formulation stability during the development of the Pheroid® formulation, as many delivery technologies are faced with stability problems. It was confirmed that stability over a wide range of APIs was improved through the control of API size, when compared to other systems (Grobler, 2009). This was shown during a stability study by Slabbert *et al.* (2011) comparing the lipophilic mefloquine entrapped in both liposomes and the Pheroid®. Following three months stability testing, the liposomal formulation size was increased from the initial measurement and with increased temperatures. The Pheroid® formulation and its entrapped API was stable for three months, with no significant observed differences among the temperatures.

The CBD-pro-Pheroid® formulations, together with the corresponding pro-Pheroid® controls, were subjected to 6 months' stability testing according to the requirement of the SAHPRA (Stability testing guidelines). Guidelines state that accelerated stability testing at 40 ± 2°C / 75 ± 5% RH needs to be executed, with three minimum sampling time points including at 0, 3 and 6 months (MCC, 2012). Additional conditions were evaluated in the current study to provide a comprehensive stability profile of the formulations. Conditions included 5 ± 2˚C, 25 ± 2˚C / 60 ± 5% RH and 30 ± 2˚C / 70 ± 5% RH. Stability can be confirmed by assessing the formulation characteristics described in section 2.3.6.

2.3.5 Analysis of Pheroid® characteristics

Analyses often performed for Pheroid® formulations include morphology, particle size distribution and zeta potential measurements to ensure optimal formulated dosage forms.

2.3.5.1 Morphology and integrity

CLSM is a useful analytical and non-destructive instrument to assess the morphology of complex pharmaceutical formulations by producing high-resolution images of samples. To ensure the visualisation of the formulations, the samples are stained with different fluorescent markers. The method then provides three-dimensional data by collecting data from multiple focal planes (Z-stack) (Collazo *et al*., 2005; Paddock, 2000). With visualisation, three main Pheroid® structures can be observed as a result of self-emulsification. This includes Pheroid® lipid-bilayer vesicles

(in the nano- and micro-range), Pheroid® microsponges (0.5 – 1.5 µm) and Pheroid® depots or reservoirs (Grobler, 2009).

2.3.5.2 Particle size distribution

The particle size distribution is often used to determine the quality of formulations with regard to solubility of the API's and delivery system particle sizes. The Mie theory is used to predict the light scattering when a particle moves through a laser beam. The angle at which a particle scatters light is measured. The particle size is reported to be indirectly proportional to the angle of the scattered light. The size distribution is then calculated and reported as d(0.1), d(0.5) and d(0.9). The mean particle size d(0.5) value is described as the size where 50% of the particles are smaller and 50% are larger than the value (Malvern, 2017). The Pheroid® vesicles in a formulation typically range between 200 nm and 2 µm (Grobler, 2009). Smaller particle sizes are optimal for oral administration, since an increased surface area results in a corresponding increased surface to volume ratio. This increases drug solvation and promotes stability (Junghanns & Müller, 2008; Williams *et al*., 2013). Thus, it is important to evaluate the long-term particle size distribution of the CBD-pro-Pheroid® formulation.

2.3.5.3 Zeta potential measurement

Zeta (electrokinetic) potential measurement is used to indicate the stability of an emulsion in the dispersion medium. Zeta potential is described as the quantitative measurement of the potential difference between the ions on the particle surface and the electroneutral region of the solution in colloidal dispersions. An emulsion with a high zeta potential (either positive or negative) indicates larger electrostatic repulsion, displaying less particle aggregation and increased emulsion stability. A zeta potential below ±25 mV may indicate coagulation or flocculation with little stability. Zeta potential values of greater than ±25 mV indicate good stability of the particles dispersed in the emulsion system (Roland *et al*., 2003).

2.4 CONCLUSION

When referring to literature, it is evident that CBD is responsible for a plethora of potential therapeutic effects and there is increasing future potential for CBD use in clinical studies. Due to the low reported oral bioavailability drug optimisation is recommended. Previous studies have confirmed that the Pheroid® is a versatile and effective drug delivery system for lipophilic drugs, with a good safety profile. Pheroid® is therefore an ideal drug delivery system for the delivery of the CBD, in order to address the difficulty in formulation and to ensure safety is maintained during the clinical trial of a CBD-pro-Pheroid® formulation.

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CHAPTER 3: THE STABILITY, ANALYSIS AND OPTIMISATION OF A CANNABIDIOL-PRO-PHEROID® FORMULATION

Chapter 3 contains the data documented during the development of the drug dosage formulation and was preliminarily written, in part, as a manuscript for the submission to the *Journal of Drug Delivery Science and Technology*, published by Elsevier. The manuscript guidelines are summarised in Annexure B, where the hyperlink to the full document is provided.

The stability, analysis and optimisation of a Cannabidiol-pro-Pheroid® formulation

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ABSTRACT

The regulation of Cannabidiol (CBD) is still highly controversial seeing that CBD is a cannabinoid isolated from *Cannabis sativa* L, from which the illegal drug cannabis is also derived. South African Health Products Regulatory Authority (SAHPRA) has recently announced that CBD products (at dosages below 20 mg/day) are exempt from scheduling due to its proven beneficial effects and the lack of associated safety concerns. This is advantageous for CBD research and product development. However, this relaxation of structured guidelines may result in an increased risk to patient safety since reporting of the product quality before human consumption is not required. This manuscript reports the accelerated stability data including the CBD potency and the characterisation of a CBD product together with Pheroid®, a beneficial drug delivery system. A PP-1 (CBD-pro-Pheroid®) formulation was evaluated and optimised to obtain a modified PP-2 formulation.

Favourable results for the PP-2 formulation were obtained as the visualisations, mean particle sizes, capsule integrity and zeta potentials were improved from formulation PP-1. From the preliminary results it was evident that the optimal storage condition for the CBD-pro-Pheroid® was potentially at 25°C/60% RH. The concentration of CBD was approximately 89% after 6 months, with an 11% decrease from baseline at all temperatures. However, at month 7 the concentration declined substantially. A loss of CBD potency increased with increased temperature, potentially due to the expiration of the pure CBD raw material. Future long-term studies are required to confirm a formulation shelf-life and to further optimise the formulation. Overall, this study added to the limited information on CBD formulation stability and also confirmed the need for adequate drug formulation and stability testing of available artisanal and commercially available CBD preparations to ensure drug quality is maintained.

Keywords: Cannabidiol; Stability testing; Pheroid®; Drug delivery system; Characterisations; Drug quality

1. INTRODUCTION

A pivotal part of the drug development process is to assess the integrity and quality of pharmaceutical drugs when introduced to different environmental factors. Drug instability can ultimately influence patient safety and the rationale for drug manufacture and distribution (Bajaj, 2012; Huynh-Ba, 2008). With the recent colossal growth of studies investigating Cannabidiol (CBD), a non-psychoactive cannabinoid of *Cannabis sativa* L., an increase in information regarding the drug's stability is expected (Grotenhermen, 2003; Zuardi, 2008). Although studies, including those of Scheidweiler *et al.* (2017) and Lee *et al.* (2012), have reported the long-term cannabinoid stability in reference to biological fluids (i.e. in blood serum, blood plasma and urine after cannabis use), this is not the case for developing CBD-formulations used during preclinical and clinical studies. Literature lacks reported data of either accelerated or long-term formal stability testing of pure CBD preparations.

The increasing evidence for the use of CBD includes the wide range of attractive therapeutic benefits associated with its use such as anti-necrotic, anti-psychotic, anti-emetic, anxiolytic, anti-convulsant and anti-inflammatory properties (Bergamashi *et al*., 2011; Pertwee, 2005; Rudroff & Honce, 2017; Zhornitsky & Potvin, 2012). The first pure CBD product (Epidiolex®; developed by GW Pharmaceuticals) was recently approved and registered at the Food and Drug Administration (FDA). Its use has shown promising results in the treatment of severe forms of epilepsy (Wise, 2018). As a prerequisite to register pharmaceutical drugs at regulatory authorities (RA), such as the FDA or the South African Health Products Regulatory Authority (SAHPRA) for the marketing of a drug, formal stability testing is required and must be executed according to The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and other applicable local guidelines. Even though a shelf-life of 18 months is reported for Epidiolex® and it is advised to discard the product 28 days after opening (Anon, 2017), additional information such as the characteristics and quality data is not published.

The plenitude of potential therapeutic benefits attributed to CBD, together with the well-established tolerability and safety of CBD products, prompted the recent relaxation of the regulation of CBD-preparations in several countries, depending on the drug source and composition. This included the European Union countries, where CBD is uncontrolled, and South Africa. CBD is currently regulated as a schedule 4 pharmaceutical drug in South Africa, based on the Medicines and Related Substances Act 101 of 1965 when indicated for therapeutic use (MCC, 2017; Pavlovic *et al.,* 2018). However, for a period of 12 months (from 15 May 2019), low dosage CBD products are exempted from scheduling in South Africa (DoH, 2019). This relaxation of CBD regulation allows CBD products to be distributed freely without specific regulatory guidelines or mandatory analytical testing, resulting in the drug characteristics, composition, stability, shelf-life and quality often not being adequately tested or reported before product

distribution (Cross, 2019; Pavlovic *et al*., 2018). This absence in structured quality testing is problematic as it is well-known that challenges exist in the formulation and successful delivery of CBD products, owing to the lipophilic nature, the intrinsic low stability and the extensive first pass metabolism of CBD (Grotenhermen, 2003; Zhornitsky & Potvin, 2012). Attention to the method of formulation is therefore necessary.

During this study, CBD was incorporated into the Pheroid® to potentially aid in the successful delivery of the water insoluble molecule. Pheroid® is a versatile drug delivery system. Its advantages in drug formulation is evident as it successfully entraps and transports hydrophobic, hydrophilic and amphiphilic compounds across biological membranes via nasal, oral or transdermal administration routes. The formulation of the Pheroid® can be altered based on the desired therapeutic outcome, the research population and different administration routes (Du Plessis *et al*., 2010; Du Plessis *et al*., 2012; Grobler, 2009). It is important to note that Pheroid®, consists of a water, an oil - (mainly of plant and essential fatty acids) and a gas (nitrous oxide) phase. For oral administration, such as described in this article, a precursor of Pheroid®, known as pro-Pheroid® was used. Pro-Pheroid® only consists of an oil and gas phase, where it is then converted to Pheroid® when introduced to an aqueous environment (Grobler, 2009).

The formulated drug is hereafter referred to as a CBD-pro- Pheroid® formulation. The objectives of this study included the formulation of an optimised *peroral* CBD-pro-Pheroid® product (test formulation) and a pro-Pheroid® formulation (control formulation). The formulations were then subjected to 6 month's accelerated stability testing and the formulation quality evaluated through analysis of the formulations by means of confocal imaging, particle size distribution and zeta potential measurements to evaluate the stability of a CBD-pro-Pheroid® formulation.

2. METHODS AND MATERIALS

2.1 Chemicals

99.14% Pure pharmaceutical grade CBD (CAS no: 13956-29-1), presented as light-yellow crystals, was purchased from Endoca (United States). The Certificate of Analysis (CoA) is provided in Annexure C. The composition of the pro-Pheroid® and the constituent ratios are currently propriety information. However, it is based on formulations described in Grobler (2009). The formulation constituents were obtained from BASF Chemicals (South Africa), Chempiro (South Africa) and Chempure (South Africa). The Vcaps® Plus capsules were provided by Capsugel (France). 32% Hydrochloric acid (HCL) was obtained from Sigma Aldrich (South Africa) and certified reference standards of (-)-CBD were purchased from LGC (South Africa). Ultrapure Milli-Q water was procured using an 18 MΩ•cm Ultrapure Milli-Q™ Water system (Synergy®

Merck). Nile Red, purchased from Molecular Probes Inc (United States of America), was used as a dye during the confocal analysis.

2.4 Formulation of study preparations

Pro-Pheroid® (PP-1 and PP-2) was formulated. Pure CBD was added to the pro-Pheroid® formulations, as depicted in Table 1, and enclosed in Vcaps® Plus capsules (size 0). All capsules were manually dosed and weighed, then sealed with a CFS 1200 capsulation machine.

The first pro-Pheroid® formulation (PP-1) was formulated, capsulated in transparent Vcaps® Plus capsules, subjected to accelerated stability testing and characterised. PP-1 was then optimised and resulted in a modified formulation; pro-Pheroid® formulation 2 (PP-2). The modified batch was then subjected to accelerated stability testing and analysed as discussed in section 2.2.3. Opaque Vcaps® Plus capsules (depicted in figure 1) were chosen for the modified formulation to potentially improve drug stability, as CBD degradation occur when exposed to light and oxygen (Trofin *et al*., 2012; Mechoulam & Hanuš, 2002). The test formulations are hereafter also referred to as CBD-pro-Pheroid® (either PP1-A or PP2-A) and the control formulations as pro-Pheroid® (either PP1-B or PP2-B).



Figure 1: Representation of the Vcaps® Plus capsules used during accelerated stability testing for formulation PP-2.

Table 1: The theoretical composition of the capsules used during accelerated stability testing.

Batch number	**PP-1**		**PP-2 (optimised)**	
Formulation	**Test (A)**	**Control (B)**	**Test (A)**	**Control (B)**
CBD (approx.)	20 mg	0 mg	20 mg	0 mg
Pro-Pheroid® (approx.)	250 µL	250 µL	450 mg	450 mg
% Concentration	8.62%	N/A	4.25% - 4.47%	N/A
Capsule	Transparent	Transparent	Opaque	Opaque

2.5 Stability testing

Accelerated stability testing, CBD assay and CBD identification were conducted at the stability chambers of the Research Institute for Industrial Pharmacy, incorporating the Centre for Quality Assurance Medicines (RIIP®/CENQAM®), a Good Laboratory Practices (GLP) compliant and ISO17025 accredited facility. The stabilities of both formulations (PP-1 and PP-2) were evaluated. Accelerated stability is based on the principle that a product stressed to extreme conditions will degrade at an accelerated rate in comparison to a product under normal conditions (Bajaj *et al*., 2012). After PP-1 was optimised to develop PP-2, the study design for stability testing was adjusted to provide supplementary stability data on the CBD-pro-Pheroid® formulation. Accordingly, the study designs for the two separate formulations differ (presented in figure 2).

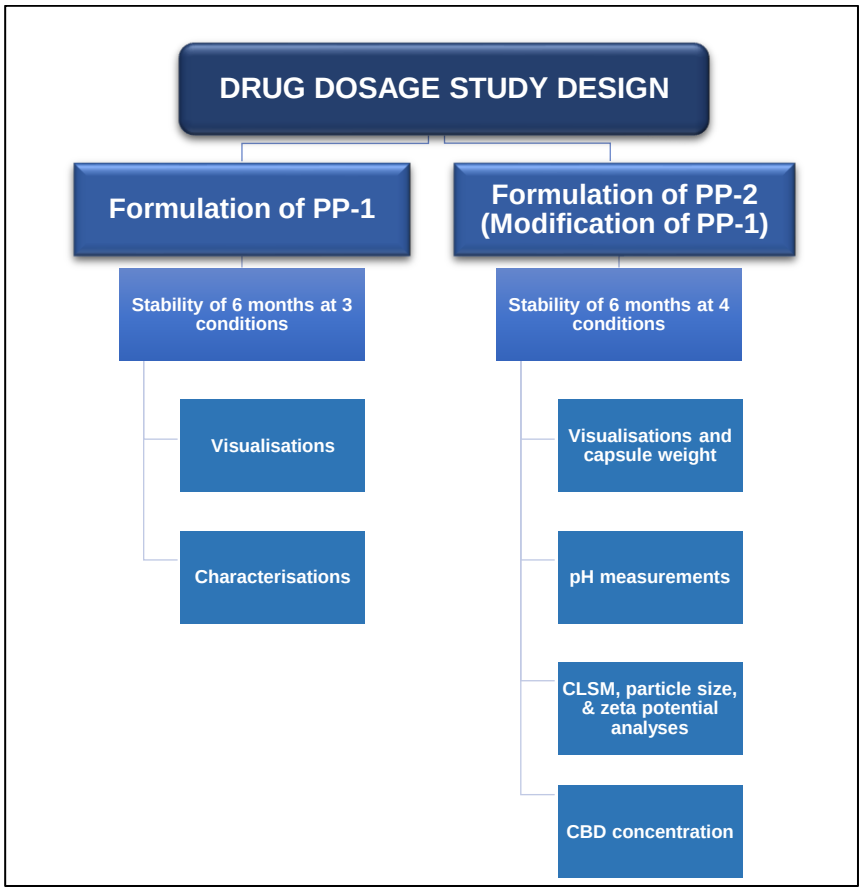


Figure 2: Representation of summarised study design following the drug formulation of both PP-1 and PP-2.

Formulations were subjected to various conditions, as provided in Table 2. Analyses of the formulation characteristics were done at baseline, 10 weeks, 3 months and 6 months on at least 1 pilot plant scale batch as recommended in the ICH GCP guidelines (MCC, 2015).

Table 2: The stability test conditions of the formulations.

Formulation	**PP1 – A (8.62% CBD)**	**PP2 – A (% CBD)**
Conditions	5 ± 2˚C 25 ± 2˚C / 60 ± 5% RH 40 ± 2˚C / 75 ± 5% RH	5 ± 2˚C 25 ± 2˚C / 60 ± 5% RH 30 ± 2˚C / 70 ± 5% RH 40 ± 2˚C / 75 ± 5% RH

As stated by Carstensens and Rhodes (2007), a pharmaceutical drug's stability can be influenced by a change in drug consistency, content uniformity, particle size and shape, pH and package integrity. Instability of the drug can result in the alteration of bioavailability and degradation of the API.

To confirm the quality and the integrity of the formulations, aside from analysis of the characteristics, each formulation was assessed for pH changes and changes in appearance (as described in section 2.4). Additionally, the PP-2 formulation was inspected for changes in weight and CBD concentration from baseline to month 6 (as described in section 2.6). The packaging was changed during formulation optimisation to potentially improve stability (figure 3(a) and (b)). Empty transparent capsules and empty opaque capsules were also included in the stability testing, during formulation optimisation, to compare the filled capsule's integrity to that of the empty capsule.

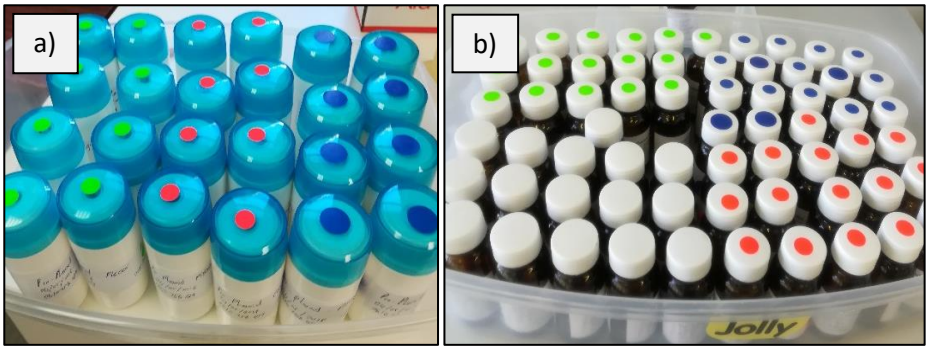


Figure 3: Representation of the capsule containers used during accelerated stability testing for (a) PP-1 and (b) PP-2.

2.5.5 Visualisation

To determine the differences in formulation and capsule integrity over a period of 6 months, the capsules were investigated for colour change, weight change, brittleness and overall shape. The formulations were also inspected for colour change and change in consistency/clarity during week 10, month 3 and month 6.

2.5.6 pH Measurements

Formulation pH measurements were included in the formulation modification to obtain supplementary data on formulation PP-2. The pH measurements were done by measuring the baseline dH_2O. The pro-Pheroid® was then added to a concentration of 4% to obtain Pheroid®, followed by sample vortex for 15 sec to ensure samples were adequately mixed. The samples were then directly measured, at ambient temperature in triplicate, by using a calibrated pH meter (Mettler Toledo, UK).

2.5.7 Analysis of formulation characteristics

Analysis of the formulations (PP-1 and PP-2) included determining the morphology, particle size distribution, zeta potential and the formulation pH to ensure that optimal formulations were developed. For consistency and successful analysis of the formulations, each sample was prepared by dilution to 4% with 0.1 N HCL, before analyses were performed (the equations are provided in Annexure D). Care was taken to minimise pre-analytical variation.

2.3.3.1 Confocal Laser Scanning Microscopy analysis

The physical characteristics of the formulations were evaluated by using Confocal laser scanning microscopy (CLSM); an analytical technique used to produce high-resolution images of fluorescently labelled samples (Paddock, 1999; Pygall *et al*., 2007). For visualisation of both the CBD-pro-Pheroid® (test formulations) and pro-Pheroid® (control formulations), 50 µL of each prepared sample was added to 1 µL freshly prepared Nile Red fluorescent marker. The samples were incubated in the dark for 15 min and dripped on a microscope slide to be investigated through CLSM. Images were captured with a Nikon D-Eclipse C1 CLSM. Image J, EZ-C1 FreeViewer and Graphpad Prism (version 6) software were used in data analysis.

2.3.3.2 Particle size distribution

The mean particle size distributions of the pro-Pheroid® formulations were evaluated by utilising a Malvern Mastersizer Hydro 2000 SM (Malvern instruments, United Kingdom). The optical system aligned automatically and the background noise was measured for 12 sec. The samples were diluted to 4% Pheroid® and manually added to the dispersant medium (dH_2O) to a light obscuration of approximately 15%, while continuously being stirred at 1500 rpm to obtain a homogenous dispersion of the formulations. The formulation particle diameter and size distribution were measured and reported in triplicate by the instrument. The main value of interest was d(0.5), which represents the diameter where 50% of the distribution is above and 50% is below this value (Rawle, 2018).

2.3.3.3 Zeta potential measurement

Zeta (electrokinetic) potential measurement is described by Roland *et al.* (2003) as the measurement of the potential difference (mV) between particle surface ions and the electroneutral region of the solution in colloidal dispersions. Zeta potential relates to the stability of particles in a formulation. A high zeta potential (either positive or negative) indicates larger electrostatic repulsion, displaying less particle aggregation and increased emulsion stability. A zeta potential of ± 25 mV may show coagulation or flocculation with little stability and values of above +25 mV or below -25 mV show good stability. At this value range particles are dispersed in the emulsion system (Roland *et al*., 2003). A Malvern Zetasizer Nano ZSP (Malvern Instruments, United Kingdom) was used to measure the zeta potential of the CBD-pro-Pheroid® and pro-Pheroid® formulations. The samples were converted to Pheroid® by diluting the sample to 4% with 0.1 N HCL to imitate the acidic conditions of the human intestine. The samples were further diluted with dH_2O to obtain a dilution of 1:500. A 1 mL of sample was inserted into a clean and undamaged Malvern cell. The same two Malvern cells were used for all measurements to decrease pre-analytical variation. Each formulation was measured in quadruplicate.

2.5.8 HPLC analysis

HPLC analysis on formulation PP-2 – Test formulation (CBD-pro-Pheroid®) and drug assays were performed by RIIP®/CENQAM® to assess changes in CBD concentration when kept at the respective conditions as described in section 2.3. The samples were analysed at baseline and month 6. Additionally, the samples were analysed at month 7, as the expiry date for the raw CBD material was reported to be at month 8. An Agilent 1100 HPLC system was utilised to analyse the optimised test formulation (PP-2) and the standard. The system consisted of a degasser, an Autosampler (set to inject 10 µL of sample and standard), a Quaternary pump, a thermostatted column oven (30°C) and a DAD detector set to record 220 nm (UV spectra 200 – 400 nm). A degassed and filtered mixture of Acetonitrile and water (Acetonitrile: H_2O; 80:20) was used as the mobile phase and was set at an isocratic flow of 1.0 mL/min. Separation was achieved with the use of a Luna C8 (A100) 250 mm x 4.0 mm 5 µm column. A standard concentration curve was obtained by preparing and analysing 1 µg/mL, 5 µg/mL, 10 µg/mL, 50 µg/mL and 100 µg/mL CBD samples. The sample solutions were quantified from the calibration curve. The CBD-pro-Pheroid® formulations (in duplicate) and standards were diluted with methanol to a concentration of 10 µg/mL and analysed. The equation to determine the change in CBD concentration from baseline, is provided in Annexure D.

2.5.9 Statistical analysis

The mean values were evaluated by one-way analysis of variance (ANOVA). Nonparametric Kruskal-Wallis multiple comparison tests were also performed with the *p*-value adjusted with a confidence value of 0.05. Differences in means (from baseline to 6 months) were considered significant if $p \leq 0.05$, using Graphpad Prism (version 6) software.

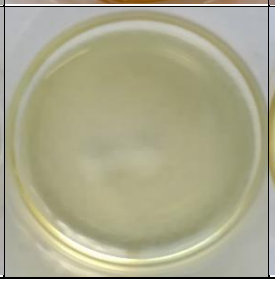
3. RESULTS AND DISCUSSION

3.1 Visualisation

3.1.1 Formulation PP-1

The visual results obtained from 6 months' stability testing at the respective conditions are discussed in this section. The PP-1 test formulation (PP1-A) and control formulation (PP1-B) are presented in Table 3, the modified PP-2 test formulation (PP2-A) and control formulation (PP2-B) are presented in Table 4, and Table 5 contains the observed data of the capsule integrity.

Table 3: The visual representation of the formulation PP-1 (CBD-pro-Pheroid®-test and pro-Pheroid®-control) when kept at the respective controlled conditions for 6 consecutive months.

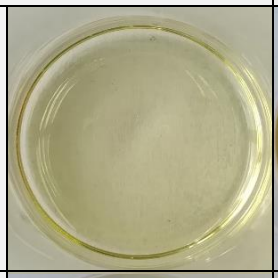
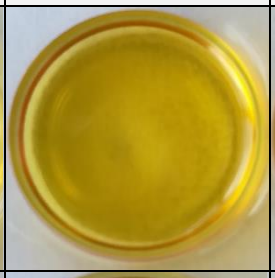
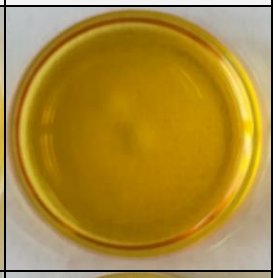
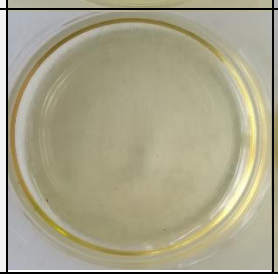
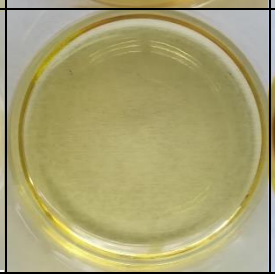
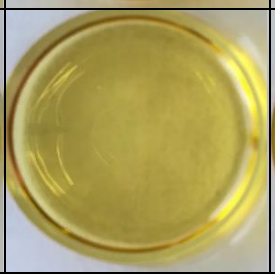
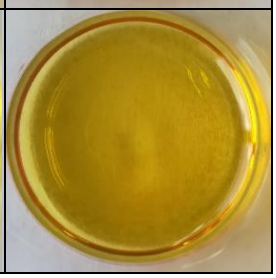
	5°C	25°C/60% RH	40°C/75% RH
Active (A)			
Control (B)			

The samples were clear and no contaminants were observed. The samples kept at 5°C for 6 months, showed promising results as no clear colour difference was observed when comparing the test formulation to the control formulation. The pro-Pheroid® control formulation darkened slightly to a light-yellow, with increased temperatures. As the baseline pro-Pheroid® products are

clear white to light yellow compared to the dark orange colour observed with the CBD-pro-Pheroid®, it is evident that the latter was more sensitive to the stability conditions. It is assumed that the CBD influenced the colour changes when the formations were kept at 25°C and 40°C, potentially due to the thermal instability of CBD. This colour change is important as commercially available oils often have a difference in reported colours as evident in a study by Pavlovic *et al.* (2018), which may indicate instability. Pavlovic *et al.* (2018) also indicated oxidation among the CBD oils. Oxidation was not measured during the current study but it may be beneficial to measure in future studies.

3.1.2 Formulation PP-2

Table 4: The visual representation of the modified formulation PP-2 when kept at the respective controlled conditions for 6 consecutive months.

	5°C	25°C/60% RH	30°C/70% RH	40°C/75% RH
Active (A)				
Control (B)				

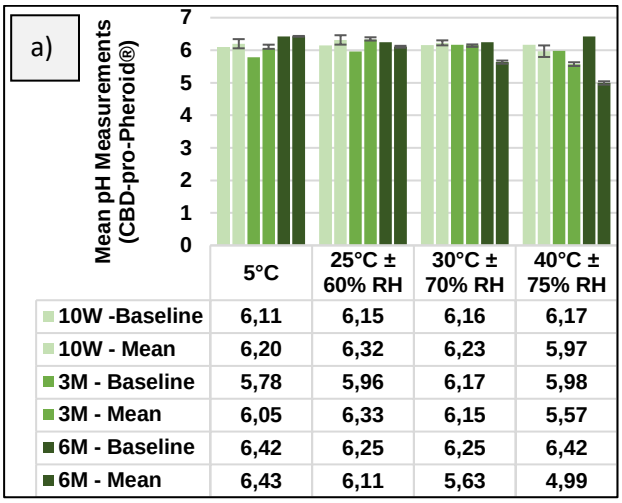
All formulations were clear and no contaminants were observed. A gradual darkening can be seen (Table 4) when comparing the visual results of the CBD-pro-Pheroid® and pro-Pheroid® formulations. Minor differences are observed between the formulations and the change is not as notable as with the unmodified formulation. It is assumed that the change in formulation rendered the CBD more stable, based on the improved appearance in comparison to Table 3. However, it would be beneficial to determine the rationale behind the colour change in future studies.

With the increased temperature, the colour also changed with the control, therefore the assumption can be made that, in contrast with Table 3, the minimal change in the modified CBD-pro-Pheroid® is potentially due to an excipient in pro-Pheroid® rather than CBD. Cassim (2009) evaluated the stability of an anti-retroviral drug (ARV) in pro-Pheroid® and also documented an increasing darker yellow colour with increased temperature and humidity. However, the study

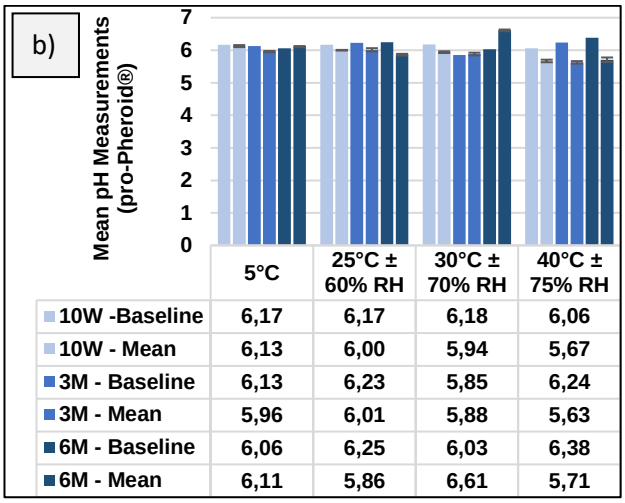
failed to utilise a pure pro-Pheroid® formulation as control, and the assumption was made that the API was potentially responsible for the change in appearance.

3.2 pH Measurements

The pH measurements obtained from the modified PP-2 test formulation (PP2-A) and control formulation (PP2-B) are presented in figure 4.



	5°C	25°C ± 60% RH	30°C ± 70% RH	40°C ± 75% RH
10W -Baseline	6,11	6,15	6,16	6,17
10W - Mean	6,20	6,32	6,23	5,97
3M - Baseline	5,78	5,96	6,17	5,98
3M - Mean	6,05	6,33	6,15	5,57
6M - Baseline	6,42	6,25	6,25	6,42
6M - Mean	6,43	6,11	5,63	4,99



	5°C	25°C ± 60% RH	30°C ± 70% RH	40°C ± 75% RH
10W -Baseline	6,17	6,17	6,18	6,06
10W - Mean	6,13	6,00	5,94	5,67
3M - Baseline	6,13	6,23	5,85	6,24
3M - Mean	5,96	6,01	5,88	5,63
6M - Baseline	6,06	6,25	6,03	6,38
6M - Mean	6,11	5,86	6,61	5,71

Figure 4: The pH measurements (mean ± SD) of the PP-2 (a) CBD-pro-Pheroid® (test – green) and (b) the pro-Pheroid® (control – blue) formulations when kept at different controlled conditions over 6 months (n = 3).

As observed in figure 4, the pH values of the MilliQ water (baselines) were below the theoretical reported pH of 6.998 at 25°C (Riché *et al*., 2006). The most probable reason for this was provided by Riché *et al*. (2006), where it is explained that counter-top pH meters are not able to accurately measure MilliQ water, as the quasi-absence of ions would enable electron transport between the sides of the pH electrode and result in a pH measurement differing from the expected value. In addition, under uncontrolled conditions, ions can dissolve readily in pure water. The presence of carbon dioxide (CO_2) in the atmosphere can dissolve in water, decreasing the pH.

To minimalise variability all measurements were performed using the same water system, buffer solutions, pH meter and environment. A single analyst calibrated the pH meter and performed the measurements. The difference in pH, measured from baseline to sample preparation, can still be attributed to the PP-2 formulations. The pH of the CBD-pro-Pheroid® formulation was slightly increased for 5°C during all measurements, for 25°C during all measurements except for month 6, and for 30°C at week 10 but decreased at month 3 and month 6. The pH for 40°C was decreased from baseline to sample preparation, for all the measurements (ranging from 5.97 ± 0.18 to 4.99 ± 0.05). This suggests a trend where an increase in temperature results in a decrease

in pH (ranging from 6.43 ± 0.01 to 4.99 ± 0.05). The pH of the pro-Pheroid® control formulation was decreased for all measurements at 25°C and 40°C.

A clear trend is not observed from the pH measurements of the control; however, the pH is decreased for month 6 at 40°C, which corresponds to the test formulation. These results are similar to a study by Slabbert *et al.* (2011) which also reported a decrease in the pH of Pheroid®. The pH of 5.88 documented during the current study was advantageous over the pH of 3.47 reported by Slabbert *et al.* (2011). Both samples were kept at 30°C for 3 months. Specific Pheroid® formulations may have an impact here, as Pheroid® can be modified for the desired study outcomes (Grobler, 2009).

3.3 Capsule integrity

Table 5: The summarised results for the CBD-pro-Pheroid® formulation when kept 40°C/75% RH for 6 consecutive months.

Formulation	Visualisation (Capsule at 40°C)	Shape	Brittleness	Colour change
PP – 1		>90% bent (severe and slightly)	Cracking >80%	Stained, corresponding to formulation colour
PP – 2 (modified)		<15% bent (Slightly)	Cracking 0%	No clear colour change as capsules were opaque

The results presented in Table 5 were subject to interpretation. Dents were observed 8 weeks after the PP-1 samples were placed on stability. The transparent capsules at 6 months were severely disfigured and most capsules did not retain their shape at 40°C. The capsules kept at lower temperatures maintained their shape. However, cracking of capsules occurred at all temperatures, but to a greater extent of those capsules kept at 25°C. The denting was presumably due to the container used, which did not properly seal (by design), and with increased temperature and humidity did not maintain the capsule integrity. The decision was made to change the containers for the optimised formulation.

With optimisation the formulation amounts were increased resulting in a filled capsule. The opaque capsule was also chosen as CBD is documented to be sensitive to light (Trofin, 2012; Mechoulam & Hanuš, 2002). The modified PP-2 formulation capsules were slightly bent at 40°C,

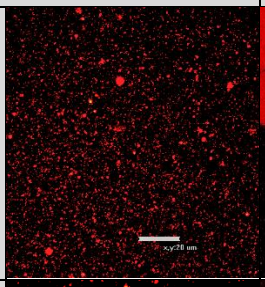
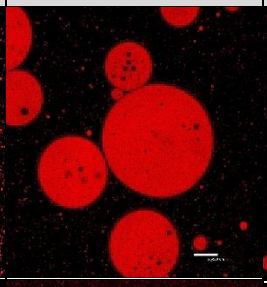
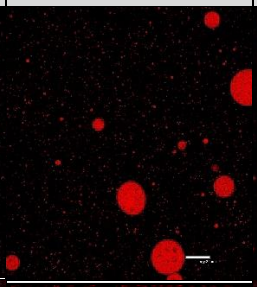
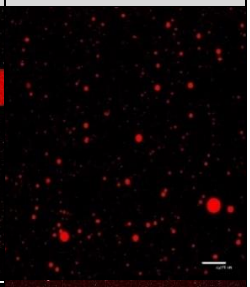
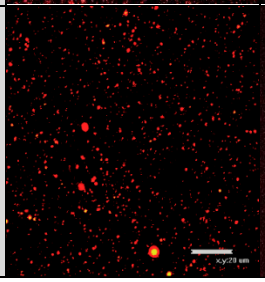
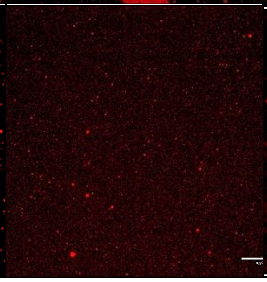
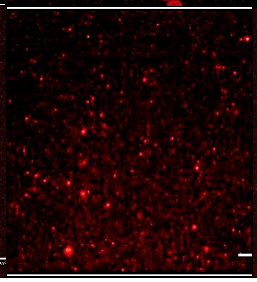
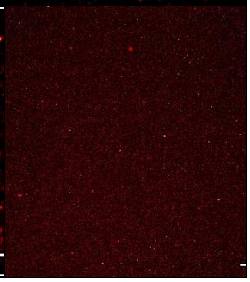
however this was improved significantly from PP-1 as this was seen in minimal capsules and only at 6 months' stability testing. No cracking was documented with the modified formulation and the empty capsules (both transparent and opaque) showed no difference from baseline. No change in container weight was observed during the 6-month period for all capsules (empty and filled) from baseline, confirming that the water content was constant during stability testing. The capsule integrity was improved based on the visualisation results.

3.4 CLSM analysis

3.4.1 Formulation PP-1

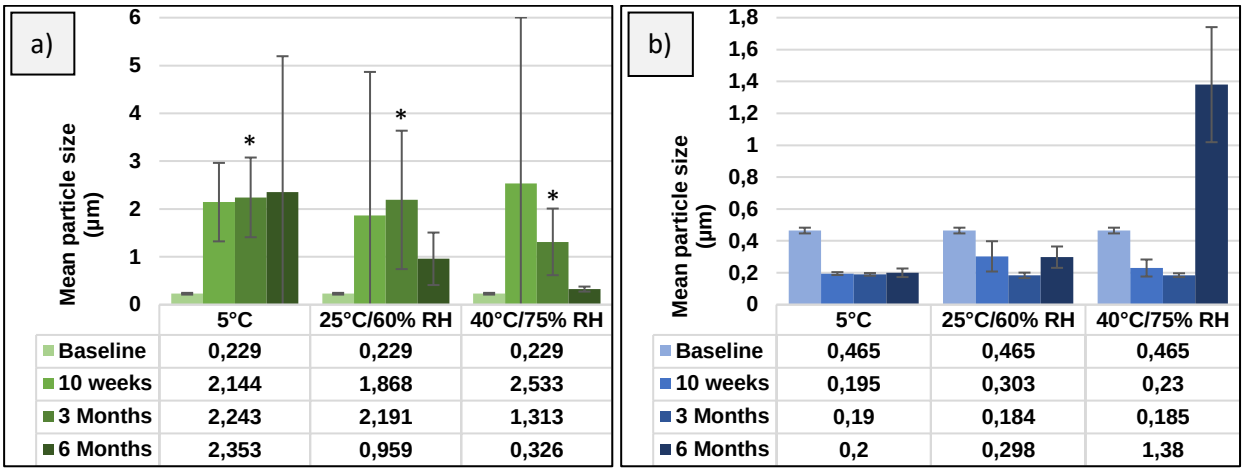
Provided in Table 6 are the confocal imaging results obtained from month 6 during stability testing at the respective conditions. The mean particle sizes calculated from the confocal analysis of the PP-1 test formulation (PP1-A) and control formulation (PP1-B) are presented in figure 5. The modified PP-2 test formulation (PP2-A) and control formulation (PP2-B) are presented in Table 7. The mean particle sizes are provided in figure 6. Supplementary data is provided in Annexure E.

Table 6: Representation of confocal imaging of the formulation PP-1 test (CBD-pro-Pheroid®) and control (pro-Pheroid®) kept for 6 months at the respective conditions, compared to the baseline. The scale bars represent 20 µm.

	Baseline	5°C	25°C/60% RH	40°C/75% RH
Test (A)				
Control (B)				

The Pheroid® vesicles are observed in both the test and the control formulation samples, where only the control sample vesicles are evenly dispersed in the medium. When comparing the baseline morphology and the corresponding 6 months' stability data of the test formulation, as presented in Table 6, clear aggregation of the API can be observed. Vesicle depots are also identified in the PP-1 (A) formulation at both 5°C and 25°C. The aggregation occurs

predominantly when the CBD-pro-Pheroid® sample is kept at 5°C and size decreases with increased temperature. This corresponds with the control, where the Pheroid® vesicles appear to decrease in size with increased temperature. This results in a sample differing significantly from the baseline. It is important to note that the images differ slightly in brightness and contrast and interpretations must be done with caution. This technique is beneficial for visualisation of the formulations.



	5°C	25°C/60% RH	40°C/75% RH
Baseline	0,229	0,229	0,229
10 weeks	2,144	1,868	2,533
3 Months	2,243	2,191	1,313
6 Months	2,353	0,959	0,326

	5°C	25°C/60% RH	40°C/75% RH
Baseline	0,465	0,465	0,465
10 weeks	0,195	0,303	0,23
3 Months	0,19	0,184	0,185
6 Months	0,2	0,298	1,38

Figure 5: Particle size (mean ± SD) of the PP-1 (a) CBD-pro-Pheroid® (test – green) and (b) the pro-Pheroid® (control – blue) formulations when kept at different controlled conditions over 6 months (n = 4). Statistical significance ($p < 0.05$) from baseline is indicated by (*).

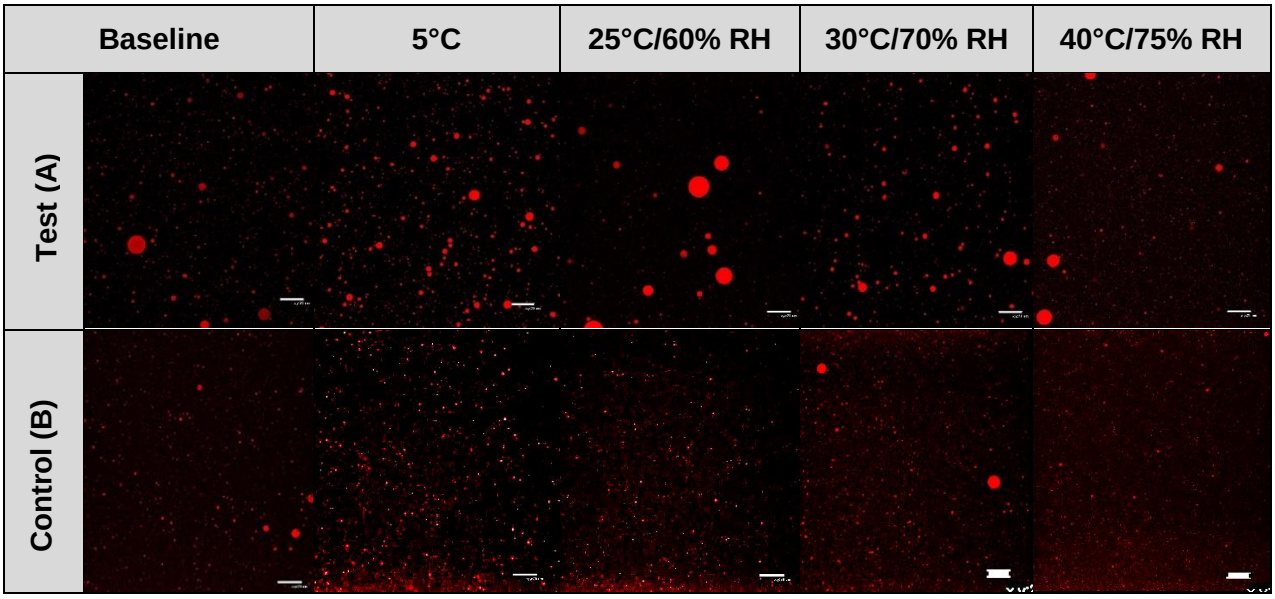
The results of the mean particle sizes are influenced by data analysis through Image J software and may be variable since changing the characteristics of the image such as the brightness, contrast between light and darkness and the sharpness, can lead to differences in the results. The analyst can also introduce variability into the results since it is challenging to obtain two results points from an identical focal plane. The images presented in Table 5 and 6 may therefore represent different areas in the sample, creating difficulty to compare the results. Multiple images were taken of one sample on different focal planes to obtain an average accounting for variability. These results should not be interpreted as quantitative but should rather be regarded as supplementary data obtained from morphology analysis. For particle size distribution, refer to section 3.5. As much care as possible was taken to use the provided image and change the same parameters for all images. Four separate images were used to obtain the mean particle size. The CBD-pro-Pheroid® mean particle size was increased at the 10-week baseline for all temperatures, while a decrease in mean particle size was observed for the control. Statistical significance ($p < 0.05$) was established from baseline in relation to all 3 of the other time intervals for the CBD-pro-Pheroid®.

The mean particle size for 40°C, at month 6, differs remarkably from month 3 for the PP-1 control. When comparing the Image J results of the pro-Pheroid® after 6 months, to the confocal imaging

(Table 6), it is clear that the control followed the same trend as the test formulation sizes decreased with increased temperature. The samples were too saturated and the vesicles too small for the Image J software to distinguish among the vesicles at 40°C, resulting in an incorrectly reported increase. In contrast, the vesicles are the smallest (as confirmed by particle size distribution presented in figure 6(b). The standard deviation (SD) of the PP-1(a) is large during all conditions and time periods in comparison to the PP-2(b) control. It appears that even after 6 months the CBD is not stable when introduced to an aqueous environment during sample analysis.

3.4.2 Formulation PP-2

Table 7: Representation of confocal imaging of the modified formulation PP-2 test (CBD-pro-Pheroid®) and control (pro-Pheroid®) kept for 6 months at the respective conditions, compared to the baseline. The scale bars represent 20 µm.



When comparing the baseline morphology and the corresponding 6 months' stability data of the test and control formulations, as presented in Table 7, minimal visual differences are observed. A notable difference is not observed among the samples when the control samples are compared to the baseline sample. The vesicles are evenly dispersed, uniform in shape and it appears that aggregation does not occur. When comparing the results of PP-1 (presented in Table 6) to the modified PP-2 results (presented in Table 7), a clear improvement of the formulation is observed.

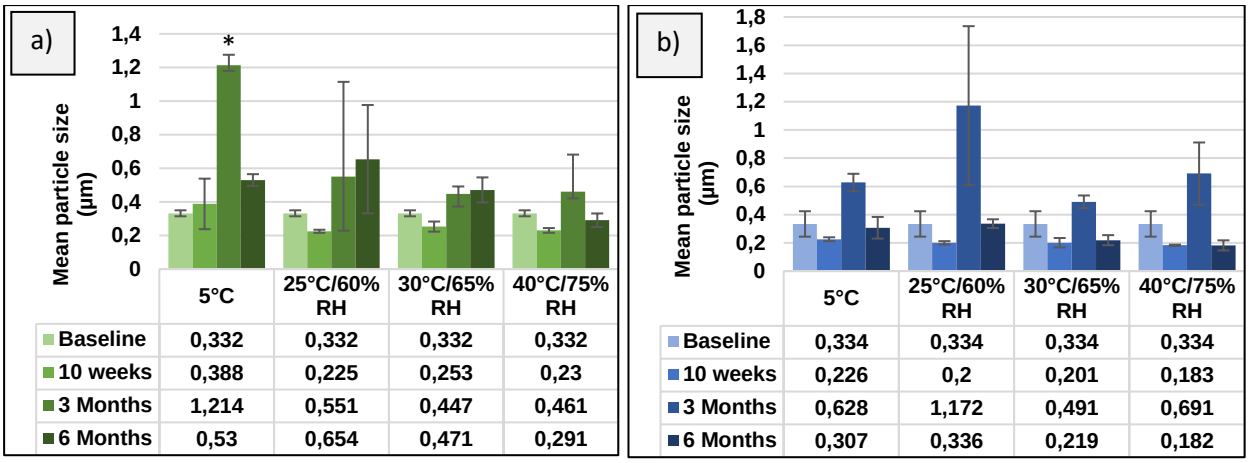


Figure 6: Particle size (mean ± SD) of the modified PP-2 (a) CBD-pro-Pheroid® (test – green) and (b) the pro-Pheroid® (control – blue) formulations when kept at different controlled conditions over 6 months (n = 4). Statistically significant difference from baseline is indicated by (*).

As observed in figure 6, the mean particle size was successfully reduced from the µm range to the nm range, evident from all temperatures. Smaller particle sizes, especially below 1 µm, are advantageous in the drug delivery process since an increased surface area results in an increased surface to volume ratio. This increases drug solvation and ultimately improves drug solubility by decreasing drug agglomeration (Junghanns & Müller, 2008; Williams *et al*., 2013). This reduction in agglomeration by reducing particle size is confirmed in Table 7, with the improvement of the confocal imaging results (Table 7). Particle size reduction is used to increase the oral bioavailability of lipophilic drugs (Junghanns & Müller, 2008; Williams *et al*., 2013). This is important as CBD has been shown to have a low oral bioavailability and poor formulation can create further challenges in drug delivery. With the introduction of the CBD-pro-Pheroid® to an aqueous environment, the pro-Pheroid® rendered the CBD formulation stable, providing potential proof of concept for the use of Pheroid® to stabilise lipophilic drugs.

Importantly, the toxicity potential of nanoparticles (structures ranging from 1 to 100 nm) has been investigated, since a reduction in size can consequently result in an increase in intrinsic toxicity. Nanotoxicity has been investigated primarily in the respiratory system. Increased reactive oxygen species (ROS) production and inflammation were documented with nanoparticles (Fu *et al*., 2014; Janroa *et al*., 2014). The sizes of the PP-2 formulations (both the test and the control formulations) are favourable as the sizes were significantly decreased (below 1 µm), yet does not comply with the nano-range criteria (below 100 nm), where nanotoxicity is documented.

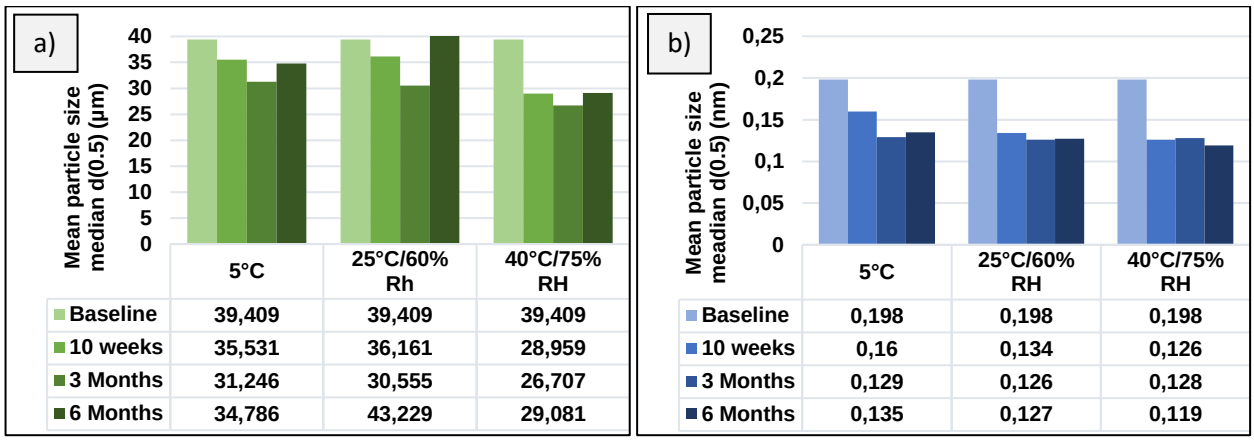
Although the particle size was improved, there are still outliers in the dataset, where the 3 months results for the both the test and the control formulations are increased from baseline. It is observed that month 3 differs from the rest of the data-set (this does not correspond to the confocal images), and it is possible that pre-analytical errors could have occurred during sample preparation or confocal analysis. According to the Kruskal-Wallis test only month 3 of the PP-2

test formulation results are statistically significantly different from the baseline, with a reported *p*-value of 0.034. However as previously stated, the results are impacted by several factors of Image J software, therefore the particle size distribution by means of Malvern Mastersizer was done to obtain quantitative results.

3.5 Particle size distribution

The mean particle size median results, obtained from baseline to month 6 during stability testing at the respective conditions, are provided in this section. PP-1 test formulation (PP1-A) and control formulation (PP1-B) are presented in figure 7. The modified PP-2 test formulation (PP2-A) and control formulation (PP2-B) are represented in figure 8. The particle size distribution curves (of month 6) are provided Annexure F.

3.5.1 Formulation PP-1



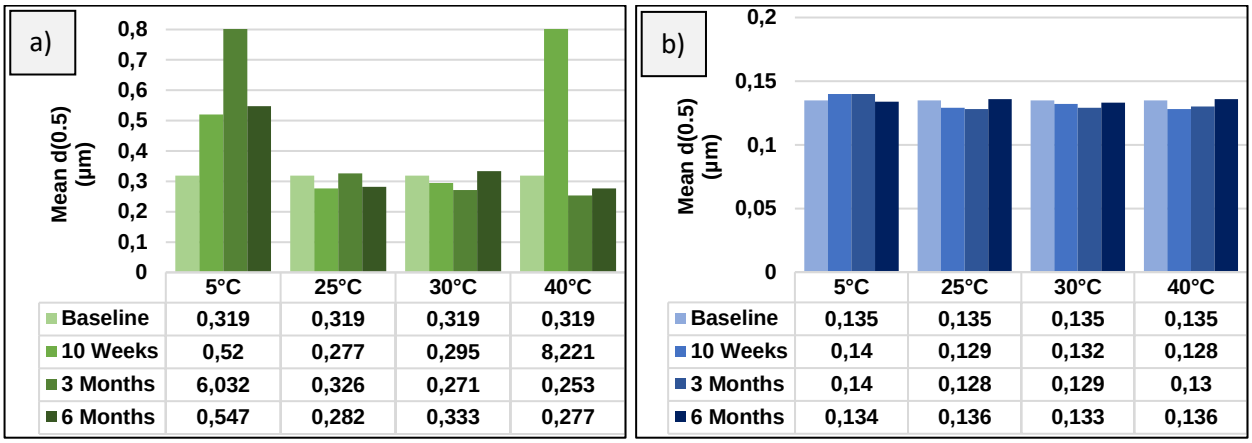
	5°C	25°C/60% Rh	40°C/75% RH
Baseline	39,409	39,409	39,409
10 weeks	35,531	36,161	28,959
3 Months	31,246	30,555	26,707
6 Months	34,786	43,229	29,081

	5°C	25°C/60% RH	40°C/75% RH
Baseline	0,198	0,198	0,198
10 weeks	0,16	0,134	0,126
3 Months	0,129	0,126	0,128
6 Months	0,135	0,127	0,119

Figure 7: Mean median of the particle size distribution of formulation PP-1 CBD-pro-Pheroid® (test - green) and the pro-Pheroid® (control - blue) formulations when kept at different controlled conditions over 6 months.

The results obtained by utilising the Malvern Mastersizer potentially show that the CBD, upon introduction to the H_2O was unstable and instantly agglomerated. A notable difference is observed between the test and control formulations. Both the test and control sample vesicles decreased in median diameter from baseline to 6 months. The diameter measured for the test formulation at 6 months ranged from 29.08 µm to 34.79 µm and the control ranged from 119 nm to 135 nm. This corresponds to the results obtained by confocal imaging (Table 6).

3.5.2 Formulation PP-2



	5°C	25°C	30°C	40°C
Baseline	0,319	0,319	0,319	0,319
10 Weeks	0,52	0,277	0,295	8,221
3 Months	6,032	0,326	0,271	0,253
6 Months	0,547	0,282	0,333	0,277

	5°C	25°C	30°C	40°C
Baseline	0,135	0,135	0,135	0,135
10 Weeks	0,14	0,129	0,132	0,128
3 Months	0,14	0,128	0,129	0,13
6 Months	0,134	0,136	0,133	0,136

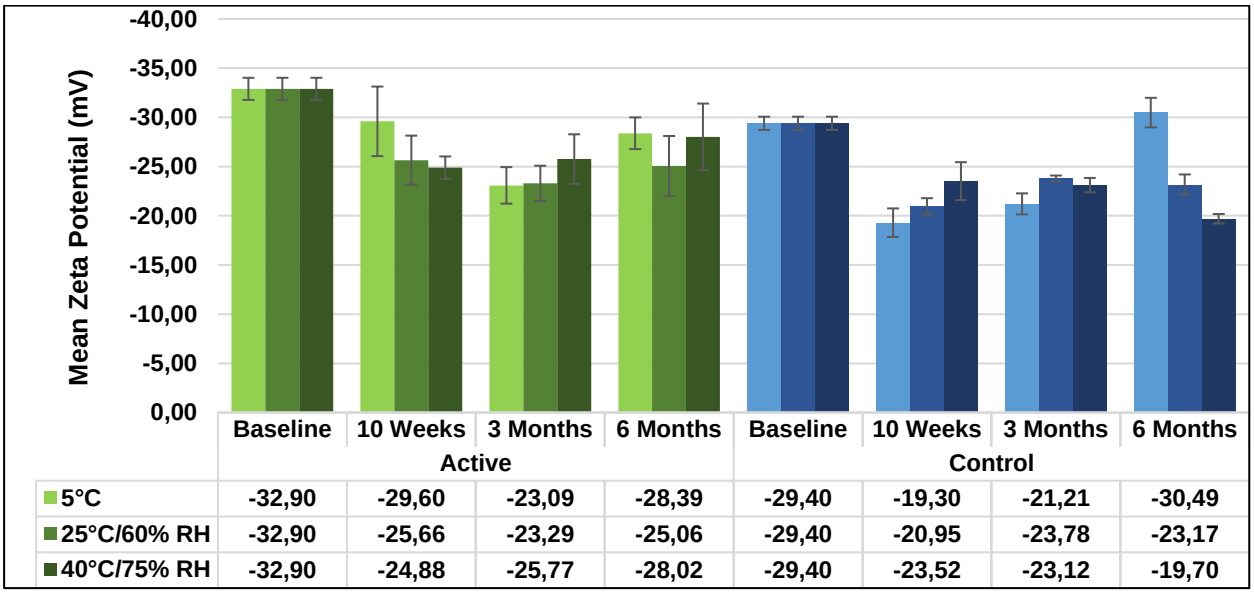
Figure 8: Mean median of the particle size distribution of the PP-2 formulation CBD-pro-Pheroid® (test - green) and the pro-Pheroid® (control - blue) formulations) when kept at different controlled conditions over 6 months.

When comparing the mean d(0.5) of the control baseline (135 nm) to 6 months (133 – 136 nm), no change was observed. This consistency shows that the pro-Pheroid® formulation was improved from formulation PP-1. The test formulation was decreased from µm to nm when the formulation was modified. Outliers were observed at month 3 for 5°C (6.032 µm) and at week 10 for 40°C (8.221 µm) during the analysis of the results. These outliers should be repeated to confirm if the formulation is unstable during the above-mentioned time intervals. No outliers were observed when the samples were kept at 25°C and 30°C.

3.6 Zeta potential analysis

This section contains the comparison for the zeta potential measurements obtained from baseline to month 6 during stability testing at the respective conditions. The PP-1 test formulation (PP1-A) and control formulation (PP1-B) are represented in figure 9 and the modified PP-2 test formulation (PP2-A) and control formulation (PP2-B) are represented in figure 10. Supplementary data is provided in Annexure G.

3.6.1 Formulation PP-1



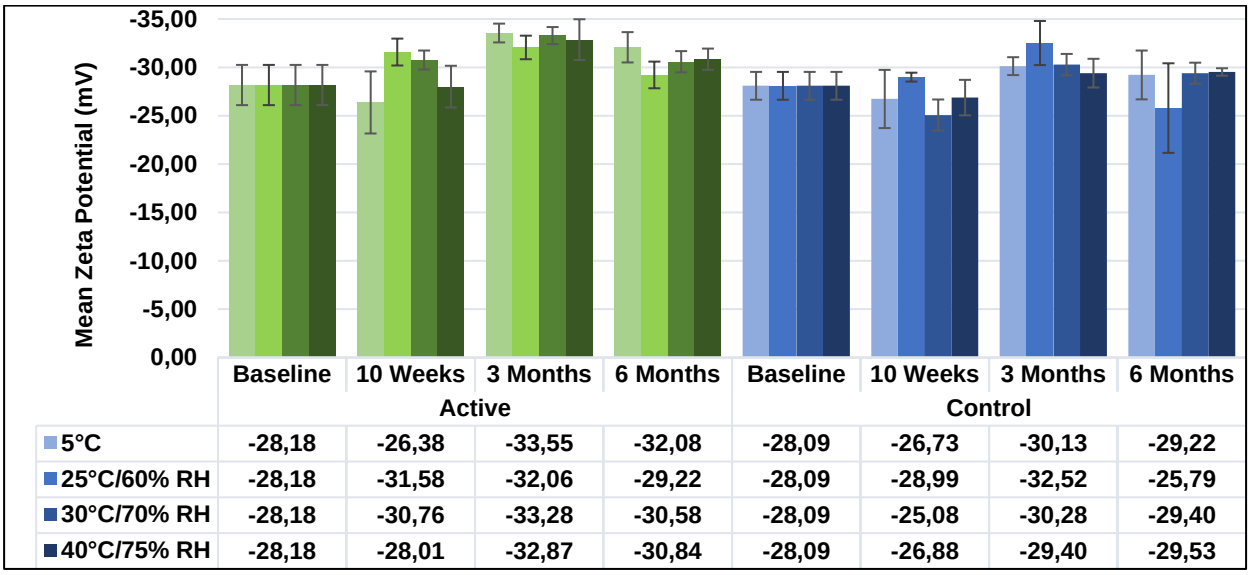
	Baseline	10 Weeks	3 Months	6 Months	Baseline	10 Weeks	3 Months	6 Months
	Active				Control			
5°C	-32,90	-29,60	-23,09	-28,39	-29,40	-19,30	-21,21	-30,49
25°C/60% RH	-32,90	-25,66	-23,29	-25,06	-29,40	-20,95	-23,78	-23,17
40°C/75% RH	-32,90	-24,88	-25,77	-28,02	-29,40	-23,52	-23,12	-19,70

Figure 9: Mean zeta potentials of the CBD-pro-Pheroid® (test –green) and the pro-Pheroid® (control –blue) formulations when kept at different controlled conditions for 6 months (n = 4).

The measured baseline zeta potential of the CBD-pro-Pheroid® (-32.90 mV) corresponds to a study by Van Wyk (2018), where a zeta potential of -32.73 mV was documented for the CBD-pro-Pheroid® formulation, despite the large reported d(0.5) of 26.94 µm. Cherniakov *et al*. (2018) reported a zeta potential of -13 mV for a CBD-PNL product with a size of 26 nm and a zeta potential of -15 mV for a CBD-piperine-PNL product with a size of 30 nm. This highlights the variability in available CBD formulations.

The documented zeta potentials, in the current study, decreased from baseline to 6 months, as can be seen with the test formulation. The zeta potentials were decreased from -32.90 ± 1.13 mV (baseline) to -28.39 ± 1.61 mV (for 5°C), -25.06 ± 3.05 mV (for 25°C/60% RH) and -28.02 ± 3.38 mV (for 40°C/75% RH). Zeta potentials also decreased for the control at all data points except for 5°C at 6 months, where the zeta potential was documented to be enhanced from -29.40 ± 0.67 mV (baseline) to -30.49 ± 1.5 mV (6 months). The test formulation, with most values reported to be below -25 mV, had an improved zeta potential when compared to the control, where the zeta potential was lowered to -19.70 ± 0.48 mV. As stated by Roland *et al.* (2003) formulations with a zeta potential above ± 25 mV show good stability since particles are dispersed in the emulsion system. The stability of the pro-Pheroid® control decreased with the increased temperatures and the stability of the CBD-pro-Pheroid® fluctuates over the 6-month period.

3.6.2 Formulation PP-2



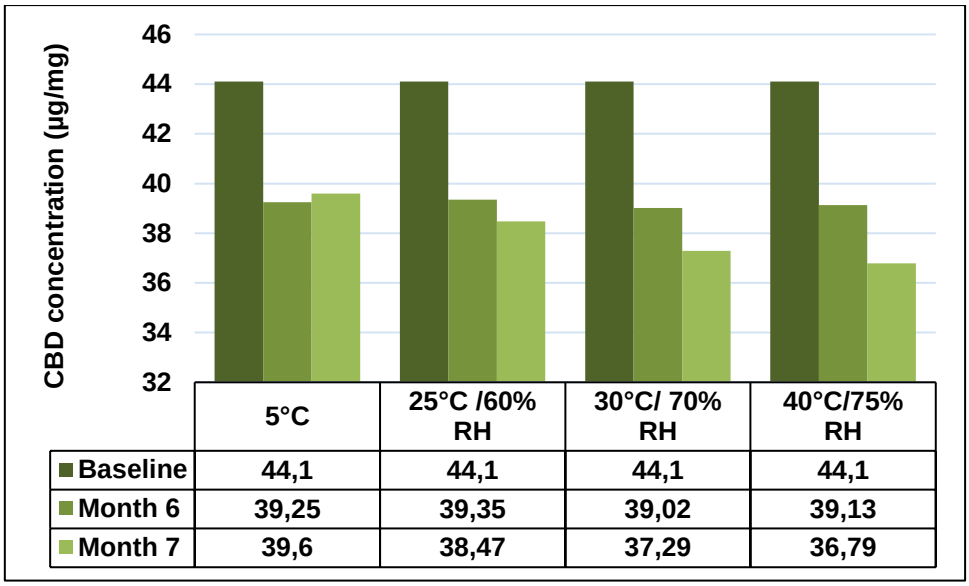
	Active				Control			
	Baseline	10 Weeks	3 Months	6 Months	Baseline	10 Weeks	3 Months	6 Months
5°C	-28,18	-26,38	-33,55	-32,08	-28,09	-26,73	-30,13	-29,22
25°C/60% RH	-28,18	-31,58	-32,06	-29,22	-28,09	-28,99	-32,52	-25,79
30°C/70% RH	-28,18	-30,76	-33,28	-30,58	-28,09	-25,08	-30,28	-29,40
40°C/75% RH	-28,18	-28,01	-32,87	-30,84	-28,09	-26,88	-29,40	-29,53

Figure 10: Mean zeta potentials of the CBD-pro-Pheroid® (test –green) and the pro-Pheroid® (control –blue) formulations when kept at different controlled conditions for 6 months (n = 4).

The modified formulation (PP-2) showed improved stability as the zeta potential fluctuated less for both the test and the control formulations over a period of 6 months. All zeta potentials were greater than -25 mV, indicating formulation stability. Zeta potentials were increased for both the test and the control formulations, with a maximum zeta potential measured at 3 months. The CBD-pro-Pheroid® zeta potentials were increased from -28.18 ± 2.08 mV (baseline) to -32.08 ± 1.57 mV (for 5°C), -29.22 ± 1.38 mV (for 25°C/60% RH), -30.58 ± 1.10 mV (for 30°C/70% RH) and -30.84 ± 1.10 mV (for 40°C/75% RH) for 6 months. The control zeta potentials increased from -28.09 ± 1.44 mV (baseline) to -29.22 ± 2.52 mV (for 5°C), -29.40 ± 1.08 mV (for 30°C/70% RH) and -29.53 ± 0.38 mV (for 40°C/75% RH). The value decreased for 25°C/60% RH from baseline to 25.79± 4.63 mV. Even with the decrease in zeta potentials, the formulation is still regarded as stable.

3.7 HPLC concentration analysis

The CBD concentration was measured in the pro-Pheroid®, by means of HPLC analysis at baseline, 6 and 7 months. The results derived from the HPLC analysis are depicted in figure 11. The equation used to determine the decrease in concentration is presented in Annexure D.



	5°C	25°C /60% RH	30°C/ 70% RH	40°C/75% RH
Baseline	44,1	44,1	44,1	44,1
Month 6	39,25	39,35	39,02	39,13
Month 7	39,6	38,47	37,29	36,79

Figure 11: Representation of the CBD concentrations (µg/mg) when kept at different controlled conditions for 6 months.

Each measurement was performed in duplicate and the averages are reported in figure 11. From the initial baseline measurement (44.1 µg/mg), the % concentration decreased to 89.00% (for 5°C), 89.23% (for 25°C /60% RH), 88.48% (for 30°C/ 70% RH) and 88.73% (for 40°C/75% RH) after 6 months. This indicates a decrease in CBD concentrations of 11%, 10.77%, 11.56% and 11.27% for 5°C, 25°C /60% RH, 30°C/ 70% RH and 40°C/75% RH, respectively after 6 months. There were no notable differences among the samples at month 6. The CBD-pro-Pheroid® kept at 25°C reportedly contained the highest CBD concentration. The samples seemingly decreased at the same rate, independent of temperature or humidity. The raw material was also measured at 6 months and resulted in a mean concentration of 97.11%, indicating a 2.03% decrease from the manufacture HPLC baseline concentration of 99.14%.

This was not the occurrence at the 7-month measurement. The CBD concentration when the CBD-pro-Pheroid® was kept at 5°C slightly increased. A gradual decrease in concentration was observed with increased temperatures, with the concentration % from baseline decreased to 89.80% (for 5°C), 87.23% (for 25°C/60% RH), 84.56% (for 30°C/ 70% RH) and 83.42% (for 40°C/ 75% RH). This indicates a decrease in CBD concentration of 10.20%, 12.77%, 15.44% and 16.58% for 5°C, 25°C /60% RH, 30°C/ 70% RH and 40°C/75% RH, respectively after 7 months stability testing. This is an unexpected decline in concentration in the capsules.

CBD is stable for a period of 24 months when stored at room temperature (25°C) in a tightly closed container, away from light based on the COA (Annexure C) and the manufacture expiry date. According to the manufacturer of the CBD, the product was to expire in 11/2019, which would have been the 8th month of stability testing. Unfortunately, the pure CBD was not measured again at 7 months. Regardless, an assumption for the significantly increased decline

observed at 7 months in concentration could be due to the expiry date, as a slight uneven discolouration (presented in figure 12) of the pure pharmaceutical product was also observed at the end of stability testing.



Figure 12: Discoloration of the pure CBD, observed approximately 2 years after manufacturing.

This could have occurred because of aliquoting from the same batch and may have resulted in instability, which cannot be confirmed here. As previously stated, stability results of CBD products are limited in literature. A clinical protocol of Epidiolex® and the Participant Information Leaflet states that the product should be discarded 30 days and 12 weeks (respectively) after opening (Anon, 2017; Anon, 2018). Anon (2017) reported a shelf-life of Epidiolex® of 18 months. The report does not provide information on the rationale behind this, or whether the product loses potency during this period. Pacifici *et al*. (2017) reported the stability of a CBD oil, when prepared from flowering buds, where ambient and refrigerated samples resulted in a 15 - 20% loss of CBD concentration within 14 days, when compared to the initial concentration. It is observed that the pharmaceutical grade product (CBD-pro-Pheroid®), reported in this study, is distinctively more stable as it retained potency over a 6-month period.

Pavlovic *et al.* (2018) measured the concentration in 14 commercially available CBD oils, where the concentrations were variable and nine out of the 14 samples notably differed from the theoretical reported amount. This emphasises the importance of not only measuring the CBD concentration, but also testing the API stability of CBD formulations for a recommended accurate shelf-life.

Taking all the results discussed during this manuscript into consideration, the optimal condition for the CBD-pro-Pheroid® formulation was presumed to be between 25°C/60% RH and 30°C/ 70% RH, based on the stability confirmed from formulation characteristics measurements. These conditions are similar to the recommended temperature to keep Epidiolex® (Anon., 2017) and the raw CBD product (COA, Annexure C).

4. CONCLUSION

It is evident, and more importantly critical, with the recent upsurge of commercially or artisanal available CBD products, that attention needs to be given to the formulation and stability testing, as many factors are known to influence the characteristics of CBD. During this study this fact has again been confirmed since a change in containers, capsules used and formulation, resulted in clear optimised results. The visualisation was improved from PP-1 to the improved PP-2 CBD-pro-Pheroid® formulation, with colour changes that were comparable to that of the control formulation. The zeta potential, particle size distribution and capsule integrity were improved with formulation optimisation. The most noteworthy characteristic change during optimisation was the decrease of reported particle sizes from the µm range to the desired nm range, which may be advantageous in improving the CBD bioavailability. Importantly, the PP-2 formulation sizes did not decrease below 100 nm, where nanotoxicity is often reported. The sizes for the formulation are desirable and within an advantageous range. These results were promising and provide the basis for future optimisations of CBD drugs. As evidenced by the results it can be assumed that the Pheroid® drug delivery system successfully stabilised the CBD in aqueous conditions. The reported stability results confirm the rationale for further use of Pheroid® in clinical studies investigating CBD.

It was noted that with measurement of the CBD concentration that the concentration measure after 6 months was still 89% of the initial measured CBD sample. However, a significant decrease in concentration was documented at 7 months. Since the pure CBD material was not measured or kept on stability, the assumption can be made that this was due to the expiry date of the pure CBD. Additional studies are needed to confirm this.

Overall the formulation was improved from the initial formulation and emphasizes that the alteration of CBD characteristics, may influence instability. This study is one of the few available, which reports on the stability and analysis of CBD products, where it also provides promising results on Pheroid® and the entrapment of CBD within the Pheroid® technology for future applications. The PP-2 formulation described in this chapter was deemed acceptable to be used in the clinical trial as described in Chapter 4.

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CHAPTER 4: A PHASE 1 CLINICAL TRIAL EVALUATING THE BIOAVAILABILITY OF A PER ORAL CANNABIDIOL-PRO-PHEROID® FORMULATION

Chapter 4 contains the data documented during the phase 1 clinical trial and was preliminarily written, in part, as a manuscript for the submission to the *European Journal of Pharmaceutical Sciences*, published by Elsevier. The guidelines for the manuscript are summarised in Annexure B.

A phase 1 clinical trial evaluating the bioavailability of a *peroral* Cannabidiol-pro-Pheroid® formulation

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ABSTRACT

Despite the increased interest in Cannabidiol (CBD), due to its plethora of potential therapeutic effects, the absorption and bioavailability of orally administered CBD are still limited. To potentially ameliorate this gap, the current study evaluated the oral administration of 20 mg CBD, together with 450 mg Pheroid®, a versatile drug delivery system. The pharmacokinetic profiles and safety associated with the use of the CBD-pro-Pheroid® formulation were compared to pure CBD in a randomised, cross-over, single-dose, phase 1 clinical trial involving 14 healthy participants. The formulations were administered in a fasted state, followed by consecutive blood samples collected over a 48-hour period. CBD plasma concentrations were determined by LC-MS/MS analysis, with a detection limit of 0.1 ng/mL.

Significant increases in CBD C_{max}, T_{max} and AUC were documented for the CBD-pro-Pheroid® formulation, where the mean AUC_{0-48} was reported as 16.03 ng/mL/h and 2.28 ng/mL/h for the CBD-pro-Pheroid® and the pure CBD, respectively. This confirms that CBD administered without a delivery vehicle results in low plasma concentrations. A high inter-subject variation was reported for the reference compound, whereas the CBD-pro-Pheroid® formulation mitigated this. The C_{max} was reported to be significantly higher in females compared to males when pure CBD was administered. This difference in the gender-based comparison of C_{max} was also ameliorated by the addition of the pro-Pheroid® formulation to CBD. The safety profile was improved with the CBD-pro-Pheroid® compared to the reference compound and no safety concerns were documented. Minor AEs were reported, with no documented SAEs. Furthermore, the majority of participants reported no perceived difference between the formulations and experienced no noticeable effects (n = 9). Even though the bioavailability of the CBD-pro-Pheroid® is favourable, as CBD is not generally administered in the pure form, additional studies are required to accurately confirm the extent of the increased bioavailability. This current clinical study supports the potential use of the CBD-pro-Pheroid® formulation for general health benefits.

Keywords: Cannabidiol; Pheroid®; Drug delivery system; Pharmacokinetics; Bioavailability; Drug safety

1. INTRODUCTION

The use of Cannabidiol (CBD), the main non-psychotropic constituent of *Cannabis sativa* L., has gained growing scientific interest over the past 15 years. This is mainly due to the broad spectrum of pharmacological effects of CBD and the favourable safety profile associated with its use (Campos *et al*., 2012; Zuardi, 2008). These effects, attributed to CBD, are under investigation in several chronic conditions such as substance abuse, multiple sclerosis (MS), schizophrenia and anxiety, among numerous others (Bergamaschi *et al*., 2011b; Hurd *et al.*, 2015; McGuire, 2017; Wade *et al*., 2004). Additional clinical research is warranted to establish the efficacy of a pure CBD product in these indications. However, multiple efficacy studies have focussed on the treatment of severe convulsions related to epilepsy and obtained confirmed beneficial results (Devinsky *et al*., 2017; Rosenberg *et al*., 2017; Welty *et al*., 2014). This recently resulted in the first pure CBD product, Epidiolex® (developed by GW Pharmaceuticals), to make headlines as it was approved by the Food and Drug Administration (FDA) for the treatment of two severe and debilitating forms of epilepsy, namely Dravet and Lennox-Gastaut syndromes (Wise, 2018).

Different CBD administration routes have been investigated in clinical trials, where CBD was predominantly administered as a sublingual spray, orally as a capsule or dissolved in an oil (Guy & Flint, 2004; Millar *et al*., 2019; WHO, 2018). Other routes have been reported such as topical, buccal, parental and inhalation administration (Chelliah *et al*., 2018; Cherniakov *et al*., 2017; Ohlsson *et al*. 1986). However, these are not primary CBD administration routes (WHO, 2018). The primary and often preferred route of administration of pharmaceutical drugs is oral, since oral administration promotes ease of use, increases safety and predictable doses to result in increased patient compliance (Anselmo & Mitragotri, 2014; Cherniakov *et al*., 2017; Millar *et al*., 2019). Nevertheless, there are still major limitations related to *peroral* CBD administered in humans. CBD encounters absorption barriers, owing to the high affinity of cannabinoids to lipids, resulting in low aqueous solubility. In addition, cannabinoids are subjected to an extensive first pass metabolism, resulting in erratic absorption of CBD (Grotenhermen, 2003; Zhornitsky & Potvin, 2012). Up to date, a single study by Ohlsson *et al.* (1986) reported the absolute CBD bioavailability in humans. Oral administration resulted in variable absorption and a low bioavailability of 6%; compared to 31% when inhaled (Grotenhermen, 2003; Millar *et al*., 2018; Ohlsson *et al*., 1986).

To address, and potentially ameliorate, these limitations of CBD oral administration*, i.e.* to increase the bioavailability, this investigation focused on the application of Pheroid® technology in the administration of CBD. Pheroid® is an effective established polydisperse drug delivery system consisting of a water-, an oil - (mainly of plant and essential fatty acids) and a gas (N_2O; nitrous oxide) phase, can be altered for different administration routes and desired therapeutic outcomes

(Du Plessis *et al*., 2010; Du Plessis *et al*., 2012; Grobler, 2009). To accommodate oral administration a precursor of Pheroid®, known as pro-Pheroid®, consisting only of an oil and gas phase, was developed (Grobler, 2009). With the addition of pro-Pheroid® to an aqueous environment, such as the gastric fluid after oral administration, Pheroid® vesicles are spontaneously produced, entrapping the active pharmaceutical ingredient (API) in the vesicles. Entrapment is followed by the transportation of the API over the biological membrane; gastric epithelial cells in the case of an oral administration (Grobler, 2008; Grobler, 2013).

Previous studies investigating the Pheroid® technology, confirmed both the safety associated with the use of the product and the potential to enhance the attributes of a wide range of hydrophilic, hydrophobic or amphiphilic compounds (Grobler, 2009; Kleynhans *et al.*, 2019; Van Wyk, 2018). This includes the enhancement of the bioavailability of certain pharmaceutical drugs, such as lumefantrine, ethambutol and artemisone (Du Plessis, 2015; Nieuwoudt, 2009; Steyn *et al*., 2011). Importantly, two studies have provided valuable preclinical information regarding the development of a CBD-pro-Pheroid® formulation. Firstly, the ability of the Pheroid® technology to increase the oral bioavailability of the highly lipophilic CBD was confirmed by the obtained favourable PK values (Cloete, 2017). Secondly, it was found that the intravenous (IV) administration of CBD-Pheroid® did not exert significant behavioural effects on the central nervous system in conscious rats (Van Wyk, 2018). These studies showed the potential benefits and provided the rationale for the use of a CBD-pro-Pheroid® formulation in clinical trials.

As a cornerstone of the drug development process, phase 1 clinical trials are critical to translate preclinical data into a clinical setting by reporting information related to the developed formulation, including the PK values and safety associated with its use (Akhondzadeh, 2016; Bergström & Långström, 2005). The promising PK and safety results, documented during both preclinical studies, supported the potential clinical use of pure CBD entrapped in Pheroid® for increased oral bioavailability. An optimised CBD-pro-Pheroid® formulation (discussed in Chapter 3) was developed. The objective of the clinical trial was to assess the PK properties and the safety of the optimised formulation by administering the formulation to healthy participants.

Millar *et al.* (2018) reviewed clinical trials where isolated/ pure CBD was administered to participants. It was evident that CBD is often administered concomitantly with Tetrahydrocannabinol (THC), the main psychoactive constituent of *Cannabis sativa* L., rather than alone. The dosages also differed largely from study to study. The maximum plasma concentration (C_{max}) and area under the curve (AUC) increased in a dose-dependent manner for lower dosages, showing a saturation effect for larger doses (ranging from 400 mg – 800 mg) (Millar *et al*., 2018). Nadulski *et al.* (2005) investigated the PK values of an acute oral administered dose of 5.4 mg in humans and documented a highly variable C_{max} of 0.30 - 2.57 ng/mL at 30 – 120 min. Guy and Robson (2004) documented a mean C_{max} of 2.47 ng/mL and a mean half-life

($t_{1/2}$) of 65.41 min after the administration of a 10 mg CBD formulation. During both studies CBD was co-administered with THC.

Even though the PK values and administered doses vary across literature, the safety corresponding to acute and chronic administered CBD dosages is well established. Dosages of up to 1600 mg/day have been safely administered, with few reported adverse events (AEs) in a study aiming to treat Schizophrenia (Bergamaschi *et al*., 2011a; Zuardi, 1995). CBD is also reportedly well tolerated in healthy volunteers, with no or minor AEs documented in several CBD-administered clinical trials. The safety profile of THC is synergistically improved with the concomitant use of CBD (Russo & Guy, 2006; McGuire *et al*., 2017). It was also indicated that physiological parameters such as heart rate, blood pressure (BP) and body temperature are not altered by the use of pure CBD (Bergamaschi *et al.*, 2011a). During the study discussed in this manuscript, the PK parameters, together with the safety associated with the oral administration of the novel CBD-pro-Pheroid® formulation, were assessed for the first time in healthy volunteers.

2. METHODS AND MATERIALS

Ethical approval for this study (Protocol no: PCDDP_2018_001) was granted by the North-West University Health Research Ethics Committee (NWU HREC) (Ethics no: NWU-00020-18-A1) on 12 October 2018. The letter of Ethical Approval is provided in Annexure A. The clinical trial was conducted in accordance with South African Good Clinical Practices (SAGCP), Good Laboratory Practices (GLP) and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. The clinical trial was executed at the MooiMed Private Hospital, Potchefstroom, North-West Province, South Africa. A narrative report is provided in Annexure L, detailing the study execution, including images of the trial site.

2.1 Study design

This was a single-dose single-centre phase 1 clinical trial with a randomised cross-over trial design. Men and women aged 18 - 55, residing in and directly surrounding the Potchefstroom area in the North-West Province of South Africa, were recruited through local advertisement (Annexure H) and attended a one-on-one information session held at the DST/NWU Preclinical Drug Development Platform (PCDDP). Each volunteer was provided with an Information Leaflet, an Informed Consent Form (ICF) and an informal Question sheet to establish baseline understanding of the study procedures and expectations. Written informed consent was obtained from each volunteer prior to study initiation. Volunteers were allowed to withdraw consent at any point during the clinical trial, without consequences or future prejudice to them.

After screening, 14 healthy participants (six females and eight males) were eligible for participation in the trial after successful clinical screening and a review of their medical history.

Each enrolled participant attended two study arms (CBD-pro-Pheroid® and CBD) and a close-out visit. After successful enrolment, participants were randomised into two groups, namely group A and B, to receive either an oral test formulation (termed CBD-pro-Pheroid®) containing pro - Pheroid® (450 mg) together with CBD (20 mg) or an oral reference compound containing pure CBD alone (20 mg). At each study arm participants arrived at MooiMed Private Hospital in a fasted state, where concomitant medication was documented in the Case Report Form (CRF) and a physical baseline examination was performed. The study was double-blinded to enable unbiased assessment of the results regarding the safety of the formulations. After confirmation of current health, participants were provided with a single opaque capsule to maintain blinding (see section 4.2.2 for capsule specifications). Participants served as their own control by receiving both the reference compound and the test formulation, by cross-over administration during two separate study visits. A three-week washout period separated the two administrations as schematised in figure 1.

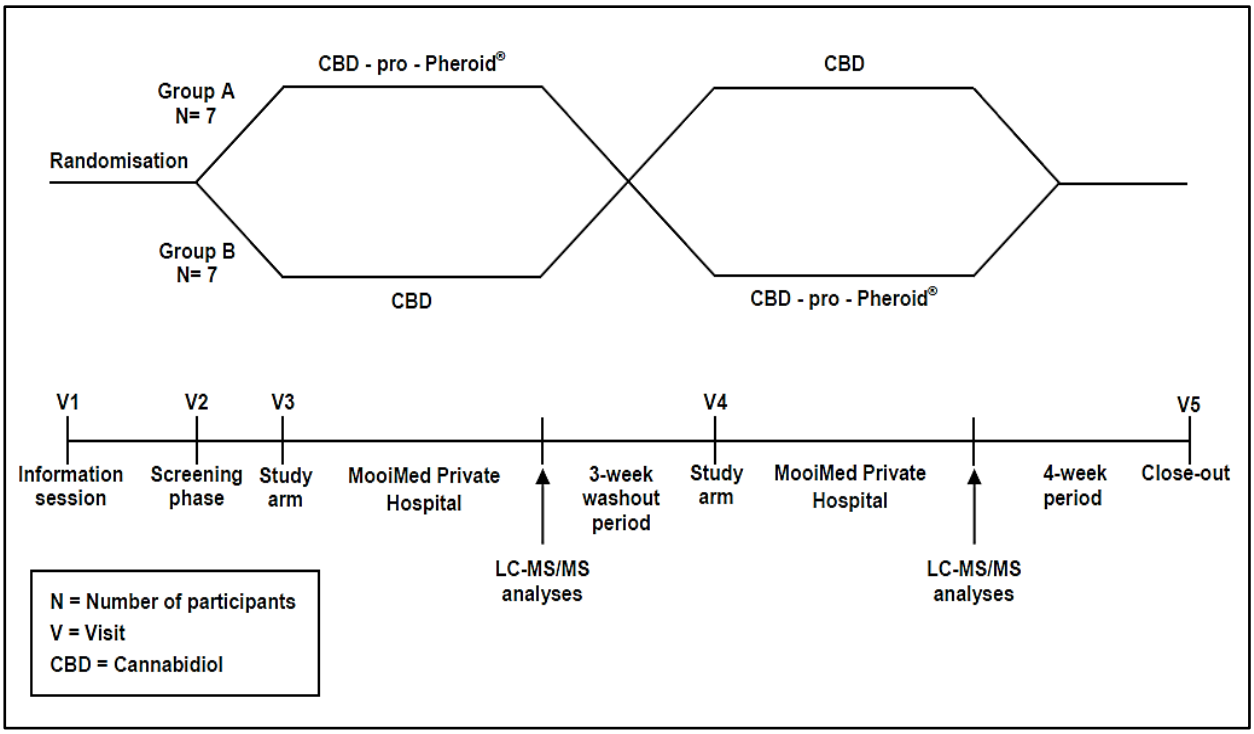


Figure 1: A schematic representation of the cross-over study design.

A washout period of 7 days would have been sufficient based on previous clinical trials investigating CBD and on the principle that a washout period should be no less than 5 elimination half-lives of the drug to ensure that no carry-over effects of preceding treatments occur (Guy & Flint, 2004; Guy & Robson, 2004; MCC, 2015). However, to allow for potential effects of drug delivery systems, such as the Pheroid®, which have been shown to result in the extended blood circulation of the pharmaceutical ingredient (Grobler, 2009), a prolonged washout period of two additional weeks was chosen for this study.

2.2 Study participants

Participants were enrolled in the clinical trial at the discretion of the Principal Investigator (PI) and the Principal Clinical Investigator (PCI). The criteria were chosen to minimise AEs and variability, and to permit detection of differences between pharmaceutical products (MCC, 2015).

2.2.1 Inclusion criteria

Volunteers were eligible for the study if they complied with the following criteria:

1. Aged between 18 and 55 years.
2. Had a Body Mass Index (BMI) ranging from 17.5 to 30 kg/m^2.
3. Healthy, as indicated by (i) a physical examination; (ii) the review of medical history and (iii) laboratory screening test results of liver enzymes including Total Protein, Alanine Aminotransferase (ALT), Gamma-Glutamyl Transferase (GGT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and total bilirubin.
4. Understood and signed the written ICF to participate in the study.

2.2.2 Exclusion criteria

Volunteers were excluded from the study if they met the following criteria:

1. Were allergic or sensitive to any component of the study formulations.
2. Donated blood within 60 days prior to study enrolment or had a history of blood/bleeding disorders.
3. BP and resting heart rate higher than 180/100 and 120 beats/min, respectively.
4. Were women who were pregnant, breastfed or planned to become pregnant during the course of the trial.
5. Used concurrent medicine that interacted with any component of the study formulations.
6. Used any enzyme modifying drugs such as cytochrome P450 inhibitors or inducers within 30 days prior to study enrolment.
7. Used CBD-based products for medicinal or recreational purposes within 30 days prior to study enrolment or were defined as a frequent cannabis or cannabidiol user (use a CBD-based product > 5 times a month).
8. Had a history of current alcohol- or drug dependence.
9. Participated in another clinical trial within 30 days prior to study enrolment.

2.2.3 Study restrictions

The participants were requested to comply with the following restrictions:

1. Avoidance of alcohol and caffeinated products 24 h prior to capsule administration.

2. Fasting from 22:00 the previous evening prior to each study visit during which capsules were administered. A blood glucose test was performed prior to each visit.
3. Refrain from using tobacco-containing products 24 h prior to capsule administration.
4. Strict avoidance of the use of cannabis or CBD-based products 30 days prior to study initiation.
5. Avoidance of enzyme modifying drugs 7 days prior to capsule administration.
6. Avoidance of pregnancy by means of at least one barrier method. Rapid urine pregnancy tests were performed prior to each capsule administration.

2.2.4 Sample size

The sample size of 14 participants was chosen based on previous randomised cross-over phase I clinical trials evaluating the bioavailability of cannabinoids, where Guy and Flint (2004) included 6 subjects per group in a phase I study design (Guy & Flint, 2004; Guy & Robson, 2004). Additional participants were added to the current study to accommodate for drop-outs and withdrawals to ensure statistical significance was maintained. No participants were withdrawn from the study by either the PI or PCI and 14 participants completed both the study arms. However, one participant was reported as a loss to follow-up and did not return to the study close-out visit.

2.3 Safety and adverse events

Safety was monitored continuously during each study arm visit. All AEs and serious adverse events (SAEs) were documented in the CRF and the Adverse Event Log and Serious Adverse Event Log, respectively. Vital signs, liver enzymes and blood chemistry were measured at baseline and 24 h after capsule administration. All equipment used during the physical examinations, including the vital sign measurements, were calibrated and the consumables were not expired and sealed where applicable. The PCI observed the participants throughout the study to ensure all concerns were promptly dealt with. An external clinical trial monitor ensured the ethical integrity of the study was maintained.

2.4 Study preparations

2.4.1 Chemicals

99.14% Pure pharmaceutical grade CBD (CAS no: 13956-29-1) was procured from Endoca (United States). API assay and identification were confirmed by the Research Institute for Industrial Pharmacy, incorporating the Centre for Quality Assurance Medicines (RIIP®/CENQAM®). Certified reference standards of (-)-CBD and CBD-d9 (Internal Standard; IS) were purchased from LGC Standards (South Africa) and Cayman Chemicals (United States),

respectively. Liquid chromatography – tandem mass spectrometry (LC-MS/MS) grade acetonitrile, methanol, water, formic acid and high-performance liquid chromatography (HPLC) grade ethyl acetate and n-hexane were all purchased from Merck (South Africa). Ultrapure Milli-Q water was obtained using an 18 MΩ•cm Ultrapure Milli-Q™ Water system (Synergy® Merck).

2.4.2 Formulation of study preparations

Pro-Pheroid® was prepared according to protocol. CBD was added to the pro-Pheroid® and enclosed in opaque size 0 Vcaps® Plus capsules, provided by Capsugel (France). Capsules (presented in figure 2) were manually dosed and weighed, followed by sealing with the CFS 1200 capsulation machine. The CBD-pro-Pheroid® formulation was subjected to formal stability testing for 6 months and characterised by means of confocal, zeta potential and particle size distribution analysis. The results are depicted in Chapter 3.

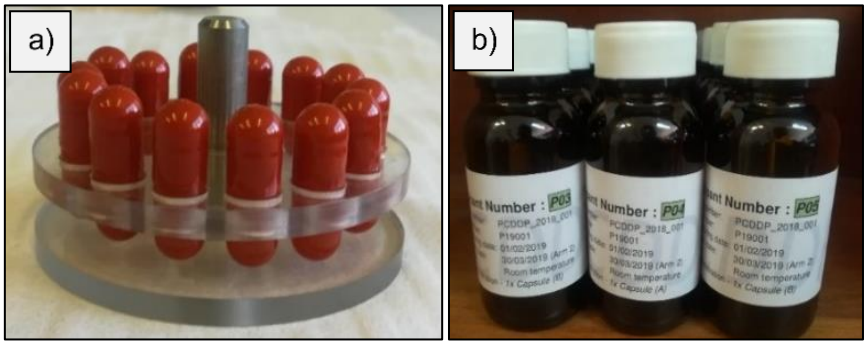


Figure 2: (a) A representation of the capsules and the (b) drug containers and labels used during the clinical trial to maintain blinding.

2.4.3 Dosage

The CBD dose of 20 mg per capsule (one capsule per participant) was chosen for the study, based on a previous study by Guy and Flint (2004) evaluating the bioavailability of cannabinoids where a dose of 20 mg reportedly produced quantifiable plasma levels. The dose was well tolerated and AEs documented with this dose were non-serious (Guy & Flint, 2004). At present, the use of pure CBD is exempt from the provisions of the Medicines and Related Substances Act 101 of 1965. This renders pure CBD unscheduled in South Africa, with limitations, where low dosages of up to 20 mg/day may be administered to promote general health (DoH, 2019). The current formulation and CBD dosage fall within this exemption.

2.5 Blood sample analysis

2.5.1 Blood sample collection for pharmacokinetic analysis

Blood samples were collected into 3 mL Ethylenediaminetetraacetic acid (EDTA) blood collection tubes at baseline, 30 min, 1 h, 1 h 30 min, 2 h, 3 h, 5 h, 8 h, 24 h and 48 h post capsule administration to obtain a complete PK profile. Care was taken to prevent haemolysis during this study to decrease the pre-analytical variability of the blood chemistry results.

A sample was recollected if visual confirmation of haemolysis could be made after the centrifugation of samples. Samples were centrifuged (utilising a Hermle Z326K centrifuge, Germany) for 10 min at 4˚C and 1500 ×g within 10 min of sample collection. Plasma was then separated into 2 mL cryovial tubes and directly placed on dry ice to maintain the integrity of the samples. Plasma samples were stored at - 80°C at the DST/NWU PCDDP until analysis. One additional 3 mL blood sample was collected into gel blood collection tubes at baseline and one at 24 h post capsule administration. The additional samples were sent to an accredited pathology laboratory for analysis of liver enzymes and blood chemistry levels.

2.5.2 Blood sample preparation

The obtained samples were provided with randomised blinding codes and prepared by a liquid-liquid extraction method. Plasma samples of 500 µL were aliquoted into separate glass tubes (13 mm × 100 mm) and spiked with 25 µL of the internal standard (IS; 0.1 µg/mL), followed by vortex-mixing for 1 min. 500 µL dH_2O was added to each tube and the samples were vortex-mixed again for 1 min. A mixture of n-hexane/ethyl acetate (80/20, v/v) was added and the tubes were placed on a roller mixer for 30 min. After the tubes were centrifuged at 1500×g for 10 min at 15°C, the upper organic layers were carefully decanted by using glass Pasteur pipettes, to new glass tubes (12 mm × 75 mm). The upper organic layers were then subjected to drying in a vacuum evaporator. The dried samples were reconstituted in 100 µL of 65% acetonitrile with 0.1% formic acid (v/v), sonicated for 3 min and vortex-mixed for an additional 3 min. The samples were transferred into amber HPLC vials and 20 µL of each was injected for analysis.

2.5.3 LC-MS/MS blood sample analysis

The LC-MS/MS analysis method was developed and validated in-house. The separations of the extracted samples were performed on Agilent 1290 Infinity HPLC system and CTC PAL HTx-xt auto sampler with a 20 µL sample loop. The analytes were separated on Phenomenex Kinetex™ C 18 column (30 mm × 2.1 mm, 1.7 µm) with a pre-column (UHPLC C18, 2.1 mm ID) at room temperature using gradient elution with water (A) and acetonitrile (B), both with 0.1% formic acid. The flow rate was 0.4 mL/min and the gradient was documented as follows: 0.0-1.0 min: linear from 70 to 95% B; 1.0-1.40 min: 95% B; 1.40-1.50 min: 95 to 70% B; 1.50-2.0 min: 70% B.

Tandem mass spectrometry was performed using a SCIEX API 4000 QTRAP mass analyser equipped with a Turbo Ion Spray source (SCIEX, Toronto, Canada) operating in electrospray ionisation (ESI) positive mode. Analyst 1.6 software was used for instrument control, data acquisition and data analysis. MultiQaunt™ 2.1 Software was used for quantification.

Detection and quantitation of the analytes were achieved using the multiple reaction monitoring (MRM) mode with a dwell time of 150 msec for all transitions. Two MRM transitions were selected for CBD, the most intense being used for quantification (m/z 315.3 → 193.1) and the other for confirmation (m/z 315.3 → 123.0). For the IS (CBD-d9), two MRM transitions were also selected; quantifier (m/z 324.3 → 202.1) and qualifier (m/z 324.3 → 268.3). The method was validated in accordance with the FDA Guidance for industry on Bioanalytical Method validation (CDER, 2001) and the European Medicines Agency on method validation (EMA, 2011). There was no interference at the retention time of CBD, as observed from six different human plasma lots. The method was linear within a range of 0.5 to 100 ng/mL, with a lower limit of quantification (LLOQ) of 0.5 ng/mL and a limit of detection (LOD) of 0.1 ng/mL. The intra- and inter-precision percent coefficient of variation (CV%) was 2.24 - 11.47% and 4.25 - 7.40%, respectively. The accuracy was within 0.40% to 8.11%. Furthermore, the CV % of the IS-normalised Matrix factor (MF) calculated from the six lots of matrices was 9.20% and 6.27% for low and high quality controls, respectively. The validation results indicated that all the parameters evaluated met the acceptance criteria.

2.5.4 Pharmacokinetic and statistical data analysis

PK parameters including $T_{1/2}$, $AUC_{0–48}$, $AUC_{0–\infty}$, C_{max}, T_{max} and the elimination rate (Ke) as well as the means and standard deviations (SD) of each parameter, were calculated for CBD after being administered in the pure form and also together with pro-Pheroid®. Concentration-time curves were used to demonstrate the mean concentrations of CBD in the plasma at a specific point in time after dosing. Data was obtained through performing a one-way analysis of variance analysis (ANOVA) using Statistica statistical software (StatSoft, Inc., 2018). Nonparametric Wilcoxon Pair T-tests and nonparametric Mann-Whitney tests were performed to establish the statistical significance between the PK values. A value was deemed significant when *p* (probability) values were < 0.05.

3. RESULTS AND DISCUSSION

3.1 Participant demographics

22 Volunteers were screened for study enrolment; 14 (8 male and 6 female) complied with the criteria and was enrolled for participation. The demographic data of the enrolled participants, based on screening results, is summarised in Table 1. None of the participants were frequent cannabis users. However, they were allowed to participate in the study, regardless of their frequency of tobacco use, where n = 6 reported daily tobacco usage. Tobacco use was nevertheless prohibited 24 h prior to and during the blood collection times. All the urine pregnancy tests were negative (n = 6) and all participant screening samples (n = 14) were void of CBD (concentration of 0 ng/mL). The obtained results were directly documented in the CRF designated to each participant.

Table 1: Participant demographic data.

Statistic (n = 14)	Age (years)	Height (m)	Weight (kg)	BMI (kg/m^2)
Mean	23.57	1.75	67.86	22.08
Median	23	1.75	62	20.93
SD	2.34	0.10	15.28	3.74
Minimum	20	1.59	50	17.72
Maximum	28	1.89	96	29.76

3.2 Capsule administration

All participants complied with the provided protocol restrictions. Following the initial blood sample collection, each participant, in a fasted state, received one allocated opaque capsule with either pure CBD (20.15 ± 0.16 mg) or CBD (20.13 ± 0.10 mg) + pro-Pheroid® (450.58 ± 0.85 mg), during each study arm, ensuring each participant took both capsules during the course of the trial. The capsule compositions are provided in Table 2. There was no significant difference between the CBD dosages of the two capsules (p = 0.67). Food was provided to participants 30 min post-administration and blood withdrawals continued in succession, as described in section 2.5.1.

Table 2: Mean composition of each capsule.

	Capsule A (Test formulation)		Capsule B (Reference compound)
	Pro-Pheroid® (mg)	**CBD (mg)**	**CBD (mg)**
Mean	450.58	20.13	20.15
SD	0.85	0.10	0.16
Median	450.20	20.10	20.10
Minimum	450	20	20
Maximum	451.9	20.3	20.5

3.3 Blood sample analysis

3.3.1 Formulation-based comparison

The mean CBD plasma concentration results for both formulations are presented in Table 3. The plasma concentration-time curves derived from the data are presented in figures 3 and 4. The PK values obtained from the blood sample analysis are provided in Table 4.

Table 3: Mean CBD plasma concentration after oral administration.

CBD-pro-Pheroid® (Test formulation)							CBD (Reference compound)						
Time (h)	**Mean (ng/mL)**	**SD**	**Median (ng/mL)**	**Min (ng/mL)**	**Max (ng/mL)**	**CV (%)**	**Time (h)**	**Mean (ng/mL)**	**SD**	**Median (ng/mL)**	**Min (ng/mL)**	**Max (ng/mL)**	**CV (%)**
0	0.00	0.00	0.00	0.00	0.00	N/A	0	0.00	0.00	0.00	0.00	0.00	N/A
0.5	0.66	0.62	0.50	0.00	1.71	93.95	0.5	0.00	0.00	0.00	0.00	0.00	N/A
1	3.00	3.21	2.06	0.12	12.46	106.99	1	0.04	0.07	0.00	0.00	0.18	165.91
1.5	3.81	2.66	2.84	0.94	9.55	69.71	1.5	0.10	0.13	0.07	0.00	0.42	124.11
2	2.45	1.81	1.79	0.59	6.36	73.90	2	0.12	0.13	0.13	0.00	0.41	105.64
3	1.35	1.05	0.87	0.37	3.44	77.77	3	0.11	0.10	0.13	0.00	0.28	85.09
5	0.59	0.40	0.48	0.24	1.66	67.05	5	0.09	0.12	0.06	0.00	0.38	123.79
8	0.32	0.19	0.27	0.15	0.93	59.37	8	0.11	0.20	0.06	0.00	0.77	176.54
24	0.15	0.04	0.14	0.11	0.23	23.57	24	0.03	0.05	0.00	0.00	0.12	198.80
48	0.11	0.05	0.12	0.00	0.16	44.21	48	0.02	0.05	0.00	0.00	0.13	254.30

CBD was not detected in the baseline samples (t = 0) for any participant. None of the participants violated the restriction of either CBD or cannabis use within the designated time period. Mean CBD concentrations were above the detection limit in the plasma samples at 30 min and 60 min post capsule administration for CBD-pro-Pheroid® and pure CBD, respectively. The obtained C_{max} ranged from 0.94 to 12.46 ng/mL among the participants, with a mean of 4.45 ng/mL, for

CBD-pro-Pheroid® and from 0 to 0.77 ng/mL, with a mean of 0.12 ng/mL, for pure CBD. Supplementary data on the individual PK values are provided in Annexure I. CBD was still present in minute amounts in the plasma samples at 48 h for both formulations. However, the collected sample count was deemed adequate to obtain complete concentration-time curves and reliable PK results as studies reported in literature measured CBD concentrations for a maximum of 12 h (Guy & Flint, 2004; Guy & Robson, 2004).

A large inter-variability of CBD concentrations can be observed among the participants in Table 3, corresponding to literature, where high inter-subject variability is documented with the administration of cannabinoids (Nadulski *et al*., 2005; Guy & Robson, 2004). CBD, in its pure form, was detected and quantifiable in the plasma after oral administration, yet the data shows a significantly larger CV %, when compared to the CBD-pro-Pheroid® formulation (p =0.0008), indicating a larger degree of variance for the pure CBD compound relative to its mean. This high variation can potentially be due to multiple samples measured, where the CBD was not detected in the plasma for several participants during the 48-h period. This confirms that CBD administered without an effective vehicle for transport is poorly absorbed after oral administration.

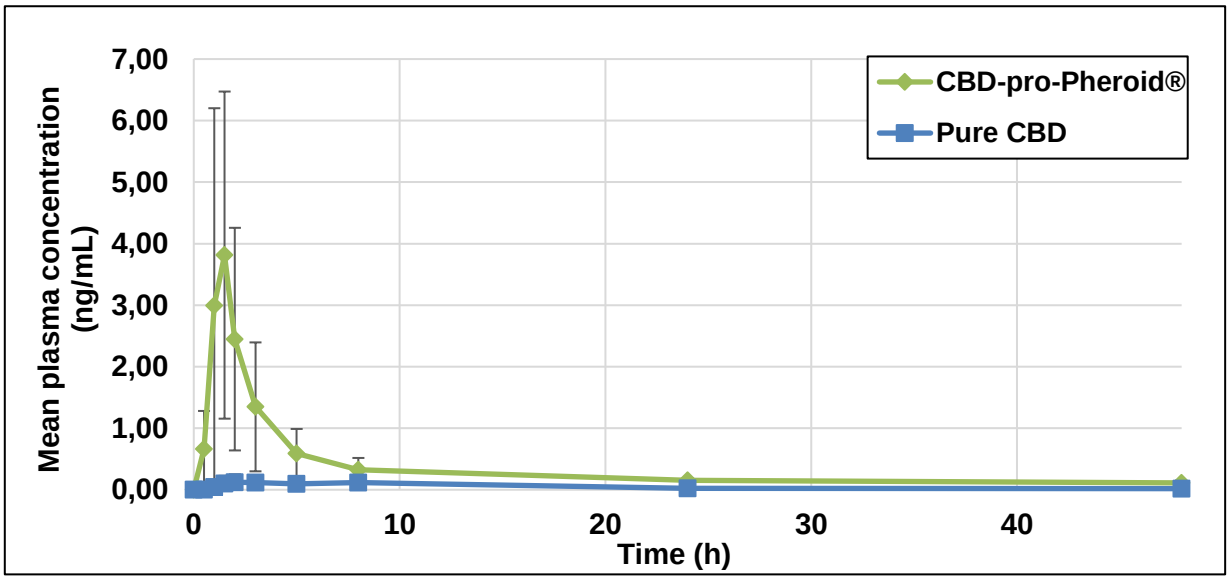


Figure 3: The CBD plasma-concentration (mean ± SD) vs. time curve following the oral administration of CBD-pro-Pheroid® and pure CBD in healthy participants (n = 14).

As presented in figure 3, the incorporation of Pheroid® into the oral administration of CBD resulted in the increased absorption of pure CBD, with a high inter-subject variability observed during the absorption phase. The plasma-concentration curve for the pure CBD is enlarged in figure 4.

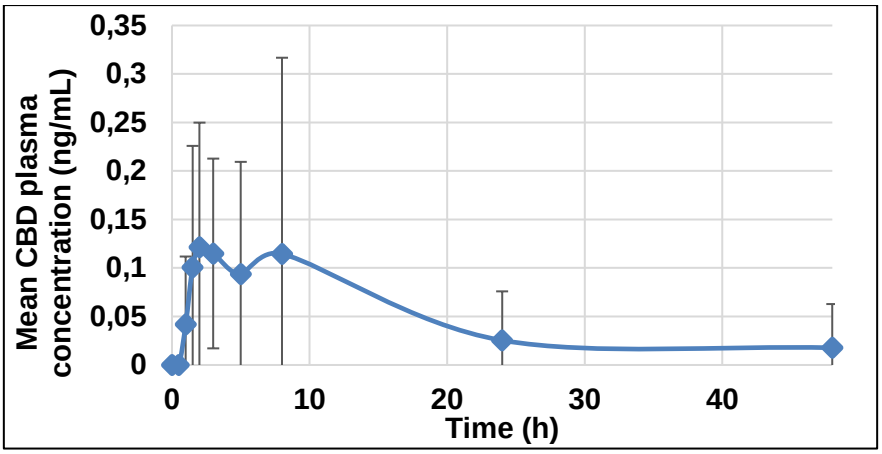


Figure 4: The CBD plasma-concentration (mean ± SD) vs. time curve after oral administration of pure CBD in healthy participants (n = 14).

Several limitations in literature exist regarding CBD formulations. Data specifically indicating the administering of CBD, without THC, is insufficient. Many of the articles documenting clinical trials involving CBD either do not report the formulation in which CBD was administered or the formulations differed from one another, restricting the comparison of the obtained results to existing data. Moreover, the use of CBD in its pure form is not often reported in literature, except for a study by Martin-Santos *et al*. (2012), which reportedly administered 600 mg pure CBD. The high dose resulted in a C_{max} of 3.4 ± 6.42 ng/mL after three hours. This is comparable to figure 3, where the oral administration of pure CBD resulted in minute plasma concentrations.

Table 4: Pharmacokinetic values obtained after oral administration of both formulations.

Statistic	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-48} (ng/mL.h)	$AUC_{0-\infty}$ (ng/mL.h)	$T_{1/2}$ (h)	Ke	AUC_{0-48} /$AUC_{0-\infty}$	C_{max} /$AUC_{0-\infty}$
CBD-pro-Pheroid® (Test formulation) n = 12								
Mean	4.45	1.25	16.03	17.09	10.99	0.07	0.93	0.24
SD	3.11	0.38	7.92	7.92	2.70	0.02	0.05	0.08
Median	4.19	1.25	13.15	14.39	10.41	0.07	0.94	0.23
Min	0.94	0.50	6.15	6.32	8.34	0.04	0.89	0.12
Max	12.46	2.00	36.30	37.30	15.57	0.11	0.99	0.40
CBD (Reference formulation) n = 6								
Mean	0.18	1.88	2.28	3.28	10.10	0.04	0.39	0.04
SD	0.23	2.32	3.10	4.68	15.83	0.07	0.44	0.06
Median	0.14	1.75	1.05	1.15	1.71	0.01	0.25	0.01
Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max	0.77	8.00	9.17	13.86	42.02	0.20	1.00	0.17

The mean C_{max} of 4.45 ± 3.11 ng/mL was reached between 1 and 1.5 h for CBD-pro-Pheroid® and a mean of 0.18 ± 0.23 ng/mL was obtained between 1.5 and 2 h for pure CBD. In a study conducted by Guy and Flint (2004) 20 mg of CBD was administered as sublingual drops to males and females. This resulted in a C_{max} ranging from 1.14 – 3.21 ng/mL with a mean of 2.05 ± 0.92 ng/mL (Guy & Flint, 2004). The mean T_{max} presented in Table 4 does not significantly differ from each other. The range of the T_{max} of pure CBD is wide (0 – 8 h) compared to the CBD-pro-Pheroid® (0.5 h – 2 h). The mean T_{max} of the pure CBD is similar to the T_{max} of 3 h reported by Martin-Santos *et al*. (2012), for the acute administration of pure CBD. The T_{max} observed for the CBD-pro-Pheroid® is comparable to literature, where Nadulski *et al.* (2005) documented the T_{max} between 0.5 h and 2 h.

It is important to note that the increase in plasma concentration did not affect the $T_{1/2}$. The reference formulation, again, was reported with a larger SD than the CBD-pro-Pheroid® formulation. The AUC_{0-48} /$AUC_{0-\infty}$ ratio provided information on the accuracy of the extrapolation of the Log concentration-curve, as this can influence the reported PK values. Where CBD was detected and the ratios were > 0.5, the data was included in the statistical analysis, since values below this resulted in an incorrectly documented enlarged extrapolation. Thus, the PK values of only 6 participants for CBD were included. The AUC_{0-48} /$AUC_{0-\infty}$ of all participants, for the test formulation, was above 0.9 and confirmed the improved results with the addition of pro-Pheroid®.

Table 5: Summarised data of the statistical significance when comparing the test with the reference formulation.

Statistic	P-value	Significant
C_{max} (ng/mL)	0.003	Yes
T_{max} (h)	0.027	Yes
AUC_{0-48} (ng/mL.h)	0.003	Yes
$AUC_{0-\infty}$ (ng/mL.h)	0.003	Yes
$T_{1/2}$ (h)	0.499	No
Ke	0.091	No

There is no significant difference between the reported $T_{1/2}$ and Ke values between the two groups (Table 5). The C_{max}, T_{max}, AUC_{0-48} and $AUC_{0-\infty}$, with the addition of pro-Pheroid®, differed significantly from that of pure CBD. This corresponds with the concentration-time curves depicted in figure 3. C_{max}, AUC_{0-48} and $AUC_{0-\infty}$ were increased and T_{max} decreased. These results follow the same trend observed in the preclinical study by Cloete (2017).

3.3.2 Sex – based comparison

Considering previous articles by Pinnow *et al*. (2009) and Sherman *et al.* (1995), which emphasise the importance of establishing safety data for various demographic groups, especially women who were previously excluded from the early developmental phases of a drug formulation, sex-based data is reported in this section for both the CBD-pro-Pheroid® formulation and the pure CBD compound. The mean CBD plasma concentration-time curves are presented in figure 5(a) and (b). The PK values obtained from the blood sample analysis are provided in Table 6.

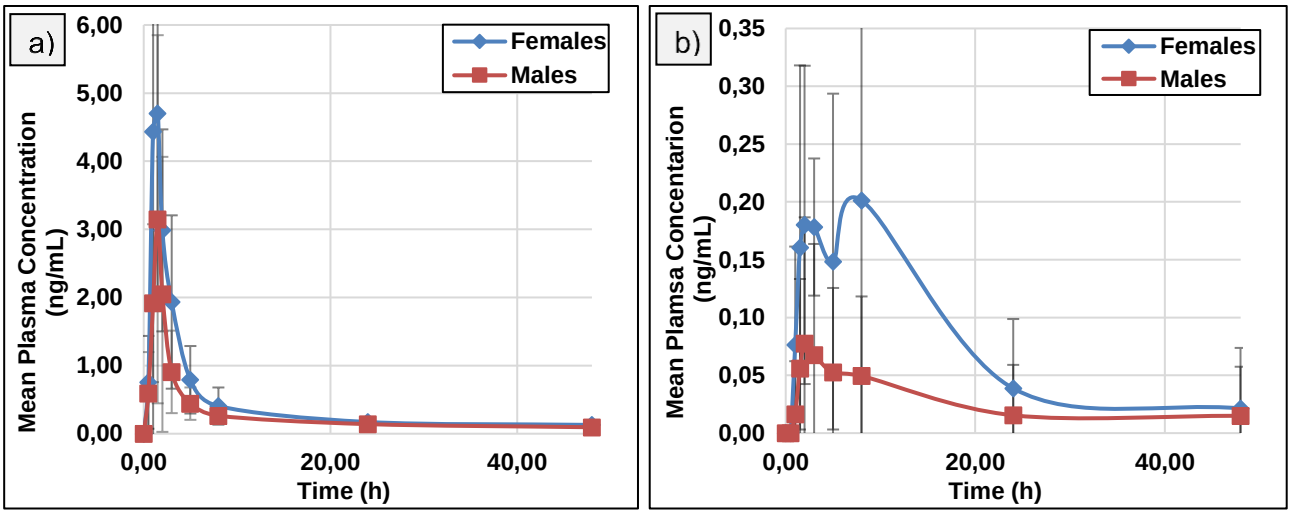


Figure 5: The CBD plasma-concentration (mean ± SD) vs. time curve after oral administration of (a) CBD-pro-Pheroid® and (b) pure CBD in male (n = 8) and female (n = 6) participants.

A study executed by Nadulski *et al*. (2005) reported a significantly higher C_{max}, AUC and T_{max} of CBD in females. This difference in CBD concentrations between the sexes can be observed, in figure 5 (a) and (b), with the administration of both formulations. An increase in CBD concentration is seen in figure 5(b), corresponding to figure 4, at the 8 h blood sample collection. As participants were in an enclosed study area for the first 8 hrs of the study, this increase is not due to the illicit usage of CBD - products unrelated to the study. Potentially, the difference in C_{max} documented among female participants resulted in a misleading increase in the CBD concentration reported after 8 hrs in females.

Pure CBD was detected in all female participant plasma (n = 6). However, pure CBD was not detectable (0 ng/mL) in the majority of males (n = 5), giving a possible rationale to the lower mean CBD concentration observed with males. The difference in CBD concentrations between the males and the females may also potentially be influenced by the weight difference between men (mean BMI is 22.52) and women (mean BMI is 21.37), seeing that the CBD drug is highly lipophilic. During this study a standardised dose was administered to males and females, not accounting for the difference in BMI or body fat composition. However, with the introduction of pro-Pheroid® to CBD,

the inter-variability between males and females was decreased. The male concentration-curve followed the same pattern as the curve of the females.

Table 6: A sex-based comparison of the pharmacokinetic values obtained after oral administration of both the CBD-pro-Pheroid® formulation and the pure CBD compound, respectively.

Formulation	CBD-pro-Pheroid®						CBD					
Mean PK values – Male Participants												
Statistic	**C_{max} (ng/mL)**	**T_{max} (h)**	**AUC_{0-48} (ng/mL.h)**	**$AUC_{0-\infty}$ (ng/mL.h)**	**$T_{1/2}$ (h)**	**Ke**	**C_{max} (ng/mL)**	**T_{max} (h)**	**AUC_{0-48} (ng/mL.h)**	**$AUC_{0-\infty}$ (ng/mL.h)**	**$T_{1/2}$ (h)**	**Ke**
Mean	3.45	1.19	12.92	13.94	11.25	0.07	0.06	0.71	0.94	1.55	6.75	0.02
SD	2.66	0.37	5.33	5.42	3.22	0.02	0.11	1.25	1.72	3.20	14.90	0.04
Median	2.17	1.25	11.17	12.66	10.41	0.07	0.00	0.00	0.00	0.00	0.00	0.00
Min	0.94	0.50	6.15	6.32	9.09	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Max	7.57	1.50	23.49	24.48	15.57	0.11	0.23	3.00	4.34	8.53	0.00	39.97
Mean PK values – Female Participants												
Mean	5.79	1.33	20.18	21.30	10.66	0.07	0.35	3.50	4.16	5.71	14.79	0.07
SD	3.40	0.41	9.34	9.21	2.03	0.01	0.26	2.60	3.80	5.68	17.58	0.09
Median	4.87	1.25	18.09	19.19	10.46	0.07	0.28	3.00	2.41	3.07	5.70	0.03
Min	2.95	1.00	11.16	12.26	8.34	0.05	0.13	1.50	0.20	0.00	0.00	0.00
Max	12.46	2.00	36.30	37.30	13.77	0.08	0.77	8.00	9.17	13.86	42.02	0.20

The mean C_{max}, for both the test formulation and the reference compound, was higher in females. The C_{max} related to the CBD-pro-Pheroid® administration ranged from 0.94 to 7.57 ng/mL for male and from 2.95 to 12.46 ng/mL for females. The C_{max} reported with the administered pure CBD was 0.00 – 0.23 ng/mL for males and 0.13 - 0.77 ng/mL for females. The C_{max} between the males and females differ significantly (p = 0.052) after pure CBD administration. The addition of pro-Pheroid® mitigated the variance gap between the male and female participants, corresponding to the concentration curves (figure 5). An investigation into the enhancement of CBD in males was done by Cherniakov *et al*. (2017), where a C_{max} mean of 2.1 ± 0.4 ng/mL and an AUC of 6.9 ± 1.3 ng/mL/h was obtained after administering a novel THC-CBD-Piperine-PNL formulation compared to Sativex® (both containing 10 mg CBD). A C_{max} of 0.5 ± 0.1 ng/mL and an AUC of 3.1 ±0.4 ng/mL/h was documented for the THC-CBD-Piperine-PNL formulation. Comparing these results to the mean AUC, in males in the current study, reported as 12.92 ± 5.33 ng/mL/h for CBD-pro-Pheroid®, the CBD-pro-Pheroid® is comparable to their successfully improved formulation.

Table 7: Statistical significance when comparing the PK values obtained from male and female participants, following oral administration of both formulations.

Formulation	**CBD-pro-Pheroid®**		**CBD**	
Statistic	**P-value**	**Significant**	**P-value**	**Significant**
C_{max} (ng/mL)	0.234	No	0.052	Yes
T_{max} (h)	0.534	No	0.857	No
AUC_{0-48} (ng/mL.h)	0.138	No	0.080	No
$AUC_{0-\infty}$ (ng/mL.h)	0.138	No	0.126	No
$T_{1/2}$ (h)	0.095	No	0.570	No
Ke	0.945	No	0.177	No

The C_{max} is significantly larger in females, compared to males, when CBD is administered. This confirms the results of the study executed by Nadulski *et al.* (2005). A considerable advantage with the use of Pheroid® is apparent, since the sex variation-gap was mitigated with the use of the Pheroid® technology.

3.4 Safety analysis

In this section, the safety associated with the use of the formulations was assessed. Complete tables with SD can be found in Annexure K. This section only reports the main data and the graphs exclusively compare the results of the two formulations, with the focus on the study arms administering the formulations.

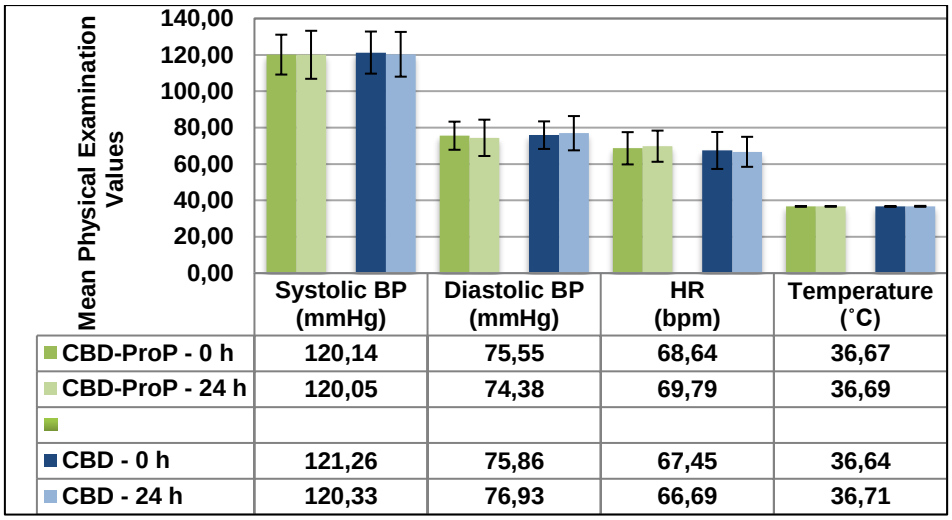
3.4.1 Physical examinations

The physical examination value means (n = 14) are reported in Table 8, depicting the change from baseline to 24 h is also given. The values are represented in figure 6.

Table 8: Mean values of the physical examination results obtained during screening (n = 14), baseline (t = 0 h) and 24 h for both study arms (n = 14), and close-out (n = 13).

Statistic	Screen	CBD-pro-Pheroid®			CBD			Close-out
		0 h	24 h	Change	0 h	24 h	Change	
Mean heart rate (bpm)								
Average	81.95	68.64	69.79	-1.14	67.45	66.69	0.76	77.56
SD	12.93	8.87	8.57	7.62	10.16	8.25	8.24	12.17
Median	83.00	70.00	70.06	-1.83	68.33	66.51	-0.12	77.67
Mean systolic blood pressure (mmHg)								
Average	121.90	120.14	120.05	0.10	121.26	120.33	0.93	114.87
SD	8.07	10.97	13.21	6.46	11.57	12.31	7.45	15.52
Median	119.83	121.40	122.69	-0.45	118.46	122.00	0.80	113.00
Mean diastolic blood pressure (mmHg)								
Average	78.14	75.55	74.38	0.28	75.86	76.93	-1.07	78.12
SD	4.81	7.74	9.99	5.27	7.58	9.42	7.57	13.35
Median	77.67	73.50	72.67	0.28	118.46	75.83	-3.33	74.30
Mean body temperature (°C)								
Average	36.84	36.67	36.69	-0.01	36.64	36.71	-0.07	36.21
SD	0.53	0.19	0.17	0.24	0.25	0.26	0.35	0.53
Median	36.90	36.69	36.69	-0.01	36.67	36.70	-0.04	36.30

The mean body temperature of participants decreased from screening to close-out. This is potentially due to seasonal changes, which occurred during the close-out visits in June 2019 in South Africa. With the exception of the measured heart rates, all calculated changes of the physical examinations from 0 h (baseline) to 24 h were decreased with the use of pro-Pheroid®. The mean heart rates for both the screening and close-out visits were higher than the visits held in the clinic, as care was taken to ensure participants were relaxed before the physical examinations prior to capsule administration. This was done to improve the accuracy of the reported results.



	Systolic BP (mmHg)	Diastolic BP (mmHg)	HR (bpm)	Temperature (˚C)
CBD-ProP - 0 h	120,14	75,55	68,64	36,67
CBD-ProP - 24 h	120,05	74,38	69,79	36,69
CBD - 0 h	121,26	75,86	67,45	36,64
CBD - 24 h	120,33	76,93	66,69	36,71

Figure 6: The physical examination results (mean ± SD) measured at baseline (t = 0 h) and at 24 h, of both the CBD-pro-Pheroid® formulation and the pure CBD.

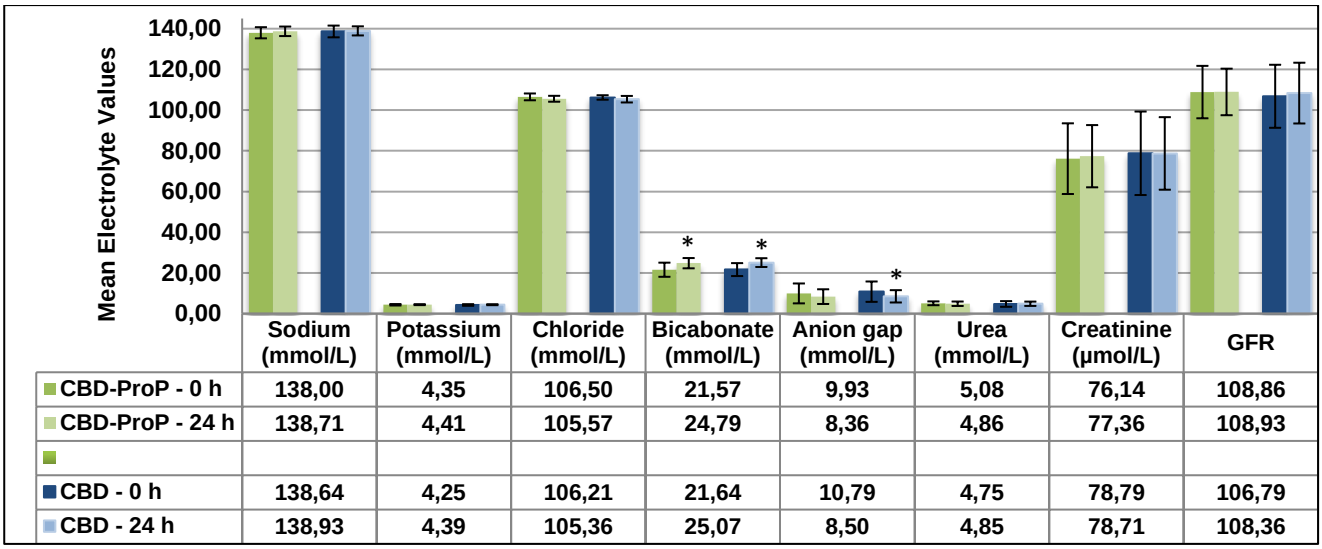
No statistically significant difference ($p < 0.05$) was documented for the measured vital signs, from baseline to 24 h, between the pure CBD and the CBD-pro-Pheroid® formulation. None of the results were clinically significant. There were also no reported AEs related to the physiological parameters. This corresponds with results obtained from Van Wyk (2018) in an animal study.

3.4.2 Blood chemistry values

The blood chemistry value means (n = 14) are reported in Table 9, depicting the change from baseline to 24 h. The values are represented in figure 7. Supplementary data is provided in Annexure J and K.

Table 9: Mean value of the blood chemistry results obtained at baseline (t = 0 h) and 24 h for the CBD-pro-Pheroid® formulation and the CBD compound.

Variable	Ref. range	CBD-pro-Pheroid®			CBD			Close-out
		0 h	24 h	Change	0 h	24 h	Change	
Sodium (mmol/L)	136 -146	138.00	138.71	0.71	138.64	138.93	0.29	139.15
Potassium (mmol/L)	3.5 - 5.1	4.35	4.41	0.06	4.25	4.39	0.14	4.60
Chloride (mmol/L)	101 - 109	106.50	105.57	- 0.93	106.21	105.36	- 0.86	105.08
Bicabonate (mmol/L)	21 - 31	21.57	24.79	3.21	21.64	25.07	3.43	27.15
Anion gap (mmol/L)	3 - 15	9.93	8.36	- 1.57	10.79	8.50	- 2.29	6.92
Urea (mmol/L)	2.1 - 7.1	5.08	4.86	- 0.22	4.75	4.85	0.10	5.46
Creatinine (µmol/L)	45 - 84	76.14	77.36	1.21	78.79	78.71	- 0.07	84.92
GFR	> 90	108.86	108.93	0.07	106.79	108.36	1.57	98.92



	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicabonate (mmol/L)	Anion gap (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)	GFR
CBD-ProP - 0 h	138,00	4,35	106,50	21,57	9,93	5,08	76,14	108,86
CBD-ProP - 24 h	138,71	4,41	105,57	24,79	8,36	4,86	77,36	108,93
CBD - 0 h	138,64	4,25	106,21	21,64	10,79	4,75	78,79	106,79
CBD - 24 h	138,93	4,39	105,36	25,07	8,50	4,85	78,71	108,36

Figure 7: The blood chemistry values (mean ± SD) measured at baseline (t = 0 h) and at 24 h, of both the CBD-pro-Pheroid® formulation and the pure CBD compound. A statistically significant hange ($p < 0.05$) from baseline is indicated by (*).

Comparing the statistical significance of the blood chemistry results between both formulations, it is documented that bicarbonate is the only value where $p < 0.05$. The values were within the reference range, where the significance is decreased when CBD is administered with pro-Pheroid®. There were no reported AEs related to the obtained electrolyte and creatinine values.

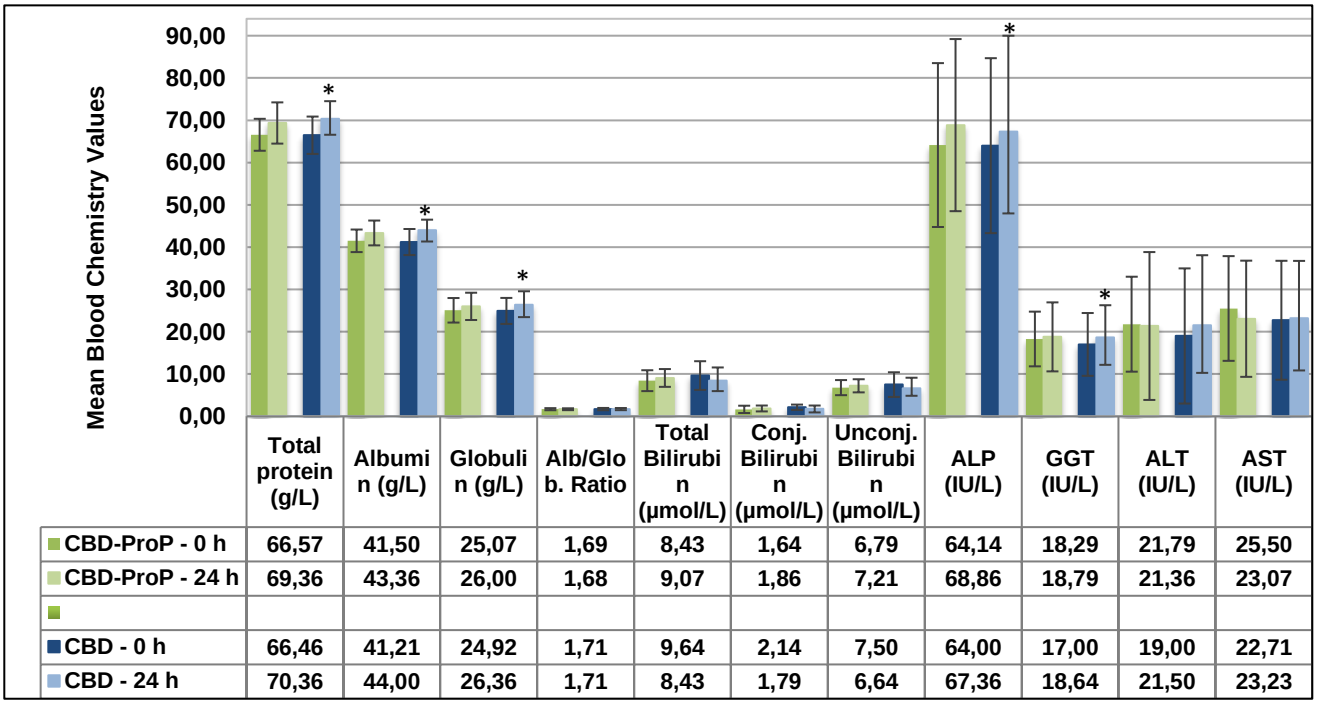
3.4.3 Liver enzyme analysis

The liver enzyme value means are reported in Table 10, depicting the change from baseline to 24 h is also given. The values are represented in figure 8. Supplementary data is provided in Annexure J & K.

Table 10: Mean value of the liver enzyme levels obtained during screening (n =14), baseline (t = 0 h) and 24 h for both study arms (n = 14) and the close-out visit (n = 13).

Variable	Ref. range	Screen	CBD-pro-Pheroid®			CBD			Close-out
			0 h	24 h	Change	0 h	24 h	Change	
Total protein (g/L)	66 - 83	74.57	66.57	69.36	2.79	66.46	70.36	3.90	72.08
Albumin (g/L)	35 - 52	44.50	41.50	43.36	1.86	41.21	44.00	2.79	44.25
Globulin (g/L)	21 - 35	30.07	25.07	26.00	0.93	24.92	26.36	1.43	27.83
Alb/Glob. Ratio	0.9 - 2.7	1.54	1.69	1.68	-0.01	1.71	1.71	0.00	1.58
Total Bilirubin (µmol/L)	5 - 21	9.92	8.43	9.07	0.64	9.64	8.43	-1.21	10.00
Conj. Bilirubin (µmol/L)	< 3.4	2.08	1.64	1.86	0.21	2.14	1.79	-0.36	1.83
Unconj. Bilirubin (µmol/L)	2 - 17	7.85	6.79	7.21	0.43	7.50	6.64	-0.86	1.56
ALP (IU/L)	30 - 120	66.79	64.14	68.86	4.71	64.00	67.36	3.36	70.15
GGT (IU/L)	< 38/ 55*	19.50	18.29	18.79	0.50	17.00	18.64	1.64	22.08
ALT (IU/L)	< 35/ 50*	20.07	21.79	21.36	-0.43	19.00	21.50	2.50	26.77
AST (IU/L)	< 35/ 50*	24.86	25.50	23.07	-2.43	22.71	23.23	0.52	23.00

*The reference values were < 35, 35 or 38 IU/L (as indicated) and < 55, 50 or 50 IU/L (as indicated) for females and males, respectively.



	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	Alb/Glob. Ratio	Total Bilirubin (µmol/L)	Conj. Bilirubin (µmol/L)	Unconj. Bilirubin (µmol/L)	ALP (IU/L)	GGT (IU/L)	ALT (IU/L)	AST (IU/L)
CBD-ProP - 0 h	66,57	41,50	25,07	1,69	8,43	1,64	6,79	64,14	18,29	21,79	25,50
CBD-ProP - 24 h	69,36	43,36	26,00	1,68	9,07	1,86	7,21	68,86	18,79	21,36	23,07
CBD - 0 h	66,46	41,21	24,92	1,71	9,64	2,14	7,50	64,00	17,00	19,00	22,71
CBD - 24 h	70,36	44,00	26,36	1,71	8,43	1,79	6,64	67,36	18,64	21,50	23,23

Figure 8: The liver enzyme values (mean ± SD), measured at baseline (t = 0 h) and at 24 h, of both the CBD-pro-Pheroid® formulation and the pure CBD compound. A statistically significant change from baseline ($p < 0.05$) is indicated by (*).

The elevation of liver enzymes, ALP and GGT, were observed with the administration of pure CBD as *p*-values were significantly ($p < 0.05$) increased from baseline to 24 h. An interventional study investigating CBD efficacy was conducted by Devinsky *et al.* (2017) and reported that four children were removed due to elevated liver enzymes. However, the CBD was administered concomitantly with epilepsy medication and in significantly higher dosages (up to 20 mg/kg) compared to the dosage used during this study. Furthermore, only ALT was documented, by Devinsky *et al.* (2017), to be increased > 3 times the upper limit of normal. Even though the changes in ALP and GGT were significant in the current study, when comparing the baseline to 24 h, all mean values were well within the reference range reported in Table 10 and still showed a favourable safety profile.

No significant difference ($p < 0.05$) was observed for any of the blood chemistry results, from baseline to 24 h, with the CBD-pro-Pheroid® formulation. It was interesting to note that values which were flagged and outside of the reference range for individual participants (but not clinically significant) at baseline, were reportedly improved at the 24 h blood sample analysis. As this is an acute study with limited participants, the significance thereof cannot be determined and no conclusion can be drawn from this. However, it would be a compelling future prospect, to include in chronic studies, as preclinical studies have confirmed that CBD successfully restores liver function in either liver toxicity or liver impairment (Avraham *et al*., 2011; Vilela *et al*., 2015).

3.4.4 Adverse events

Summarised in Table 11 are the adverse events reported after oral administration of the respective formulations.

Table 11: Summarised reported adverse events associated with the formulations.

No.	Adverse Event	CBD N (Participant count)	CBD-pro-Pheroid® N (Participant count)
1	Headache	3	4
2	Hematoma at IV site	1	0
3	Sinus	1	0
4	Stiffness/pain at puncture site	1	1
5	Tiredness/Solemness /Relaxation	1	4

No SAEs occurred and only minor AEs were reported (n = 16) throughout the clinical trial. When comparing the reported AEs, a clear difference is only evident with regards to tiredness/ relaxation, where one participant reported tiredness with the use of pure CBD and four participants reported the AE with the use of the CBD-pro-Pheroid® formulation. The participants described a feeling of tiredness /relaxation /solemness. Devinsky *et al.* (2017) reported several

AEs when a dosage of 20 mg/kg/day was administered to children for a period of 14 weeks. The reported AEs included lethargy and solemness, where AEs were mainly elevated with dosage reduction. However, as mentioned above, the study was executed concomitantly with standard epilepsy medication and at notably higher dosages compared to the current study.

As participants were not certain of the exact effects felt during the current study, many reported the AE and it was mainly interpreted as tiredness by the medical staff. This effect could potentially be the anxiolytic effect attributed to the use of CBD products, as CBD has been used in the treatment of anxiety (Blessing *et al*., 2015).

Headaches were the most commonly reported AEs during this clinical trial. Insufficient clinical data is available to establish if these reports were related to CBD use, or circumstantial (Lochte *et al*., 2017). A study by Naftali *et al.* (2017) documented headaches as an AE to the clinical trial. However, the headaches from the CBD-group in their study were not clinically significant when compared to the headaches reported from the placebo group. Here the headaches reported were also comparable for the test formulation and reference compound.

3.4.5 Participant feedback

13 Participants completed a questionnaire, at the close-out visit, regarding how the CBD-pro-Pheroid® formulation was perceived by them. Nine participants reportedly would use the formulation again, where six participants reported no effects, two felt calm or relaxed and one would use it as a sleeping aid. One participant was unsure if he/she would use the formulation again as he/she felt no effects. An additional two participants reported that they would not use the formulation again, since one felt no effects and the other reportedly felt too relaxed. According to these anecdotal results the majority of participants felt no effects with 20 mg CBD. There was also no clear difference in the perception between male and female participants (two females and two males reported the calming/relaxed/sleep aid effects). Participants could not differentiate between the two drugs (based on taste and effects), supporting the idea that, should the formulation be used on a daily basis, it could either produce no effects or a calming effect. However, chronic studies are required to confirm this. A single participant reported that the drug can be used as a sleeping aid, where three reported feeling calm/relaxed. This corresponds with the above assumption that the reported AEs could have been the anxiolytic effect. Further investigation is warranted to confirm the exact description and extent of the reported tiredness/solemness/relaxation AE. It is evident from the blood sample analysis, participant feedback and AEs that the CBD-pro-Pheroid® formulation had favourable reported safety.

4. CONCLUSION

The advantages associated with the use of the Pheroid® drug delivery system in combination with CBD are clear considering that the safety of Pheroid®, with GRAS status excipients, has been tested and confirmed. When the PK properties of the pure CBD with the CBD-pro-Pheroid® formulation is compared, it is evident that the Pheroid® successfully entrapped and transported the CBD. This in itself is the primary rationale and confirmation for the future development of the CBD-pro-Pheroid® product. The clinical research results resemble the results from preclinical studies investigating the PK and safety of the CBD-pro-Pheroid®.

When comparing the current CBD-pro-Pheroid® formulation to existing literature, favourable results are observed. The gap in literature produces challenges to the confirmation of the bioavailability (as a percentage), as information on a pure CBD-product is still largely lacking and the need for a standardised CBD-formulation is evident. This study is important as the results obtained contribute to the development of a standardised formulation. Furthermore, no safety concerns arose from the study execution. No SAEs were observed and only minor reported AEs. Interestingly, the use of the Pheroid® decreased variance between the male and female groups, albeit difference between the groups was not observed. The Pheroid® can thus potentially be used in the development, manufacturing and distribution of a standardised pharmaceutical grade CBD-formulation.

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CONFLICT OF INTEREST

The authors had no conflict of interest to declare.

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CHAPTER 5: STUDY CONCLUSION

5.1 STUDY OUTCOMES

Due to the future potential, and the uncertainty surrounding CBD drugs, it was important to ensure a high-quality, safe and deliverable drug was formulated, while gathering supplementary information on the long-term stability of the drug and the drug delivery system. The zeta potential, the particle size and the morphology characteristics were improved for the CBD test formulation from PP-1 (CBD-pro-Pheroid®) to the optimised PP-2 (CBD-pro-Pheroid®). The original formulation was unstable following 6 months' stability testing and significant changes from baseline were observed even with the benefits of the Pheroid® technology. This emphasises the need for adequate CBD formulation, even with artisanal preparations, since regardless of the obtained PK and efficacy results, if the CBD-oil quality and stability is not confirmed, unstable formulations may be provided to patients, increasing the risks to said patients. This should be a consideration during the decision to extend the exemption on CBD scheduling, currently in South Africa. Although the exemption provides many benefits to progress the CBD research field, guidelines (even if less stringent) will still be beneficial.

The phase one clinical trial also provided insights in both the optimised CBD-pro-Pheroid® and the Pheroid® technology. CBD availability in the plasma was significantly increased, the T_{max} decreased and the difference in $T_{1/2}$ was not deemed statistically significant. In addition, the study reported the differences between male and female PK values, seeing as the majority of studies preferred to include exclusively male participants. Interestingly, the C_{max} for females were significantly higher when compared to the males, when pure CBD was administered. This corresponds to the current literature. However, the pro-Pheroid® drug delivery system ameliorated this, since no significant change was observed when the CBD-pro-Pheroid® drug was administered. Regardless of the limitation discussed above, the stability, increased bioavailability and lack of serious adverse events, together with the potential to decrease inter-subject variation between male and females, provide evidence that the Pheroid® is promising for an improved CBD formulation. Reflecting on the study objective of assessing bioavailability, and the individual study aims, the study was successfully executed and provides information for future considerations.

5.2 STUDY LIMITATIONS

An important study limitation was encountered during the study execution. The administration of pure CBD confirmed that CBD alone is unable to cross the relevant biological membranes, restricting bioavailability. Pro-Pheroid® significantly increased the bioavailability relative to the control. However, since it is not common practice to provide pure CBD to patients, the extent to which the bioavailability was increased could not be established as the relative bioavailability could not be accurately confirmed. The conscientious decision was made to use the pure

pharmaceutical grade CBD as a reference compound, as participant safety was the highest priority during the study. The bioanalytical method did not prove to be sensitive enough for the pure CBD and often resulted in levels below the level of detection.

To mitigate this limitation, future studies will need to include a positive control rather than the pure CBD for comparison, consisting of commercially available products, which have not been approved as pharmaceutical grade products by a regulatory authority. This approach produces multiple challenges, posing risks to participants, as the reported contents of the commercially available CBD products have been shown to vary significantly from the labels, with adequate safety data and origin lacking for most products (Bonn-Miller *et al*., 2017). A superior method of determining the extent to which the bioavailability is increased, will be to obtain Epidiolex® (the only registered CBD product) or to administer CBD dosages corresponding to literature and comparing the obtained PK values (similar to this study). However, the literature containing data on isolated CBD, independent and separate from THC, is currently limited.

5.3 FUTURE RECOMMENDATIONS

During the study it was noted that future studies will benefit from the following:

During the drug dosage formulation:

- Include a positive CBD-preparation comparator.
- Use analytical methods to measure the oxidation and photosensitivity of CBD and pro-Pheroid® formulations on stability to determine the reason for the slight colour change.
- Improve the sensitivity of the LC-MS/MS method if pure CBD is evaluated.
- Determine the shelf-life of the product and confirm stability beyond 6 months.
- Form a discussion group about good practices to follow in terms of the conditions to which CBD products should be subjected to for stability testing.

During the clinical trial:

- Assess the influence of participant body fat percentage on the PK parameters.
- The current study design included measurement of vital signs at baseline and 24 h. It may be beneficial to measure the physiological parameters of participants, after drug administration, more frequently during each study arm.

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Prof AF Grobler
Pharmaceutical sciences
NWU/DST Preclinical Drug Development Platform

12 October 2018

Dear Prof Grobler

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00020-18-S1

Kindly use the ethics reference number provided above in all future correspondence or documents submitted to the administrative assistant of the Health Research Ethics Committee (HREC) secretariat.

Study title: A phase 1 clinical trial evaluating the bioavailability of peroral cannabinoid-Pheroid® formulations

Study leader: Prof AF Grobler

Student: S Erasmus-23172215

Application type: Single study

Risk level: High (monitoring report required three-monthly)

Expiry date: 31 October 2019 (monitoring report is due at the end of February, April, July and October annually until completion)

You are kindly informed that after review by the HREC, Faculty of Health Sciences, North-West University, your ethics approval application has been successful and was determined to fulfil all requirements for approval. Your study is approved for a year and may commence from 12/10/2018.

Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation. A monitoring report should be submitted two months prior to the reporting dates as indicated i.e. annually for minimal risk studies, six-monthly for medium risk studies and three-monthly for high risk studies, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECMonitoring@nwu.ac.za. Annually, a number of studies may be randomly selected for an internal audit.

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. These requests should be submitted to Ethics-HRECApply@nwu.ac.za with a cover letter with a specific subject title indicating, "Amendment request: NWU-XXXXX-XX-XX". The letter should include the title of the approved study, the names of the researchers involved, the nature of the amendment/s being made (indicating what changes have been made as well as where they have been made), which documents have been attached and any further explanation to clarify the amendment request being submitted. The amendments made should be indicated in yellow highlight in the amended documents. The *e-mail*, to which

you attach the documents that you send, should have a *specific subject line* indicating that it is an amendment request e.g. "Amendment request: NWU-XXXXX-XX-XX". This e-mail should indicate the nature of the amendment. This submission will be handled via the expedited process.

Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form to Ethics-HRECIncident-SAE@nwu.ac.za. The *e-mail*, to which you attach the documents that you send, should have a specific subject line indicating that it is a notification of a serious adverse event or incident in a specific project e.g. "SAE/Incident notification: NWU-XXXXX-XX-XX". Please note that the HREC, Faculty of Health Sciences has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

The HREC, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely

Prof Wayne Towers
HREC Chairperson

Prof Minrie Greeff
Ethics Office Head

Included in Annexure B is summarised requirements for the preparation of manuscript for publications in the indicated journals. To access the full Author Information Pack the hyperlink is provided below.

CHAPTER 3 GUIDELINES FOR AUTHOR INFORMATION PACK

https://www.elsevier.com/journals/journal-of-drug-delivery-science-and-technology/1773-2247?generatepdf=true

DESCRIPTION .
The Journal of Drug Delivery Science and Technology is an international journal devoted to drug delivery and pharmaceutical technology. The journal covers all innovative aspects of all pharmaceutical dosage forms and the most advanced research on controlled release, bioavailability and drug absorption, nanomedicines, gene delivery, tissue engineering, etc. Hot topics, related to manufacturing processes and quality control, are also welcomed.
IMPACT FACTOR .
2018: 2.606 © Clarivate Analytics Journal Citation Reports 2019

ADDITIONAL INFORMATION
The authors should ensure that they have written entirely original works, and if the authors have used the work and/or words of others, this has been appropriately cited or quoted. An author should not in general publish manuscripts describing essentially the same research in more than one journal of primary publication. Plagiarism in all its forms as well as submission of the same manuscript to more than one journal concurrently constitutes unethical publishing behaviour and are unacceptable. Thus, the Letter of Submission should explain in one paragraph the originality and significance of the submitted work. The manuscript will not be processed without this information.
ARTICLE STRUCTURE
Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.
Material and methods
Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.
Results
Results should be clear and concise.
Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature. Conclusions The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.
Abstract
A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.
Keywords
Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

CHAPTER 4 GUIDELINES FOR AUTHOR INFORMATION PACK

https://www.journals.elsevier.com/european-journal-of-pharmaceutical-sciences

DESCRIPTION

.

The journal publishes research articles, review articles and scientific commentaries on all aspects of the pharmaceutical sciences with emphasis on conceptual novelty and scientific quality. The Editors welcome articles in this multidisciplinary field, with a focus on topics relevant for drug discovery and development More specifically, the Journal publishes reports on medicinal chemistry, pharmacology, drug absorption and metabolism, pharmacokinetics and pharmacodynamics, pharmaceutical and biomedical analysis, drug delivery (including gene delivery), drug targeting, pharmaceutical technology, pharmaceutical biotechnology and clinical drug evaluation. The journal will typically not give priority to manuscripts
focusing primarily on organic synthesis, natural products, adaptation of analytical approaches, or discussions pertaining to drug policy making. Scientific commentaries and review articles are generally by invitation only or by consent of the Editors. Proceedings of scientific meetings may be published as special issues or supplements to the Journal.

Manuscripts submitted to the Journal are only accepted on the understanding that (a) they are subject to editorial review (generally by two independent reviewers); (b) they have not been, and will not be, published in whole or in part in any other journal; (c) the recommendations of the most recent version of the Declaration of Helsinki, for humans, and the European Community guidelines as accepted principles for the use of experimental animals have been adhered to.

IMPACT FACTOR

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2018: 3.532 © Clarivate Analytics Journal Citation Reports 2019

GUIDE FOR AUTHORS

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INTRODUCTION

Manuscripts submitted to the journal are accepted on the understanding that: (1) they are subject to editorial review, (2) they have not been and will not be published in whole or in part in any other journal and (3) the recommendations of the most recent version of the Declaration of Helsinki, for humans, and the European Community guidelines as accepted principles for the use of experimental animals, have been adhered to. *The European Journal of Pharmaceutical Sciences* will, therefore, only consider manuscripts that describe experiments which have been carried out under approval of an institutional or local ethics committee.

Types of Paper

Research articles

The *European Journal of Pharmaceutical Sciences* publishes research articles in the multidisciplinary field of pharmaceutical sciences, with a focus on topics relevant for drug discovery and development. More specifically, the Journal publishes reports on medicinal chemistry, pharmacology, drug absorption and metabolism, pharmacokinetics and pharmacodynamics, pharmaceutical and biomedical analysis, drug delivery (including gene delivery), drug targeting, pharmaceutical technology, pharmaceutical biotechnology and clinical drug evaluation.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places:

1. A summary declaration of interest statement in the title page file (if double-blind) or the manuscript file (if single-blind). If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted.
2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyrightholder.

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Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

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Role of the funding source

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Funding body agreements and policies

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Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

PREPARATION

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Results Results should be clear and concise. Text, Tables and figures must show minimal overlap, and must be internally consistent. *Discussion* This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for Tables and figures: Table A.1; Fig..

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• ***Corresponding author.*** The manuscript must be submitted by the person who is in charge of correspondence at all stages of the editorial process, production, and post-publication. Ensure that phone numbers (with country and area codes) are provided, in addition to the e-mail address (preferably an institutional e-mail address) and the complete postal address. Contact details of the other authors must be kept up to date by the corresponding author.

• ***Author names and affiliations.*** Where names may be ambiguous (e.g., a double name, or possible confusion about first/last names), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lowercase superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and the e-mail address (preferably an institutional email address) of each author.

• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are optional yet highly encouraged for this journal, as they increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling

Abbreviations of units of measurements and other terms are as follows:

The isotope mass number should appear before the atomic symbol, e.g., [3H]noradrenaline, [14C]choline. Ions should be written: Fe3+, Ca2+, Mg2+. The term absorbance (A) is preferred to extinction or optical density. For abbreviations not included in this list consult: *Units, Symbols and Abbreviations, A Guide for Biological and Medical Authors and Editors*, 1994 (The Royal Society of Medicine, London), ISBN 0-905958-78-0, or *Scientific Style and Format. The CBE Manual for Authors, Editors, and Publishers*, 6th edn. (Cambridge University Press, Cambridge), ISBN 0-521-47154-0.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g. lab technicians, statisticians, colleagues providing help preparing the manuscript).

Formatting of funding sources

clear. The use of the appropriate prefix is essential. Use of the generic name alone without number according to *Enzyme Nomenclature*, rev. edn. (Academic Press, New York, NY, 1984) should

be quoted the first time the enzyme is mentioned.

Artwork

Image manipulation

Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, this journal is applying the following policy: no specific feature within an image may be enhanced , obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.
• Ensure that color images are accessible to all, including those with impaired color vision.

Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A. caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit Tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number Tables consecutively in accordance with their appearance in the text and place any Table notes below the Table body. Be sparing in the use of Tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in Table cells.

ENDOCA

https://www.endoca.com

Certificate of Analysis

Quality
GMP

Cannabidiol (CBD)
Product Name

Sample Reference:	Batch 678
Analysis Method:	100.SM.00004
Analysis ID:	81445
Date of Analysis:	2017.05.03

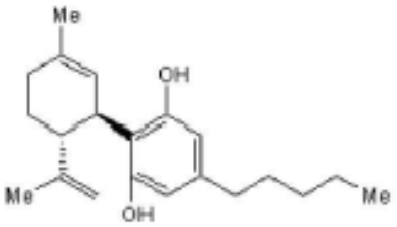
Manufacture: UAB "ENDOBIOTECH"
Tunelio g. 60
Kaunas, LT-44405

Date of Manufacture: 02.05.2017

Product Name: (-)-Cannabidiol
Pharmaceutical grade
CAS Number: 13956-29-1
IUPAC Name: 2-[(1R,6R)-3-Methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol

1. PHYSICAL AND CHEMICAL PROPERTIES

Batch Molecular Formula:	$C_{21}H_{30}O_2$
Batch Molecular Weight:	314.46 g/mol
Physical Appearance:	White to colored crystalline powder
Storage:	Protected from light and tightly closed at ≤ 25 °C
Solubility:	Practically not soluble in water, soluble in organic solvents
Molecular Structure:	

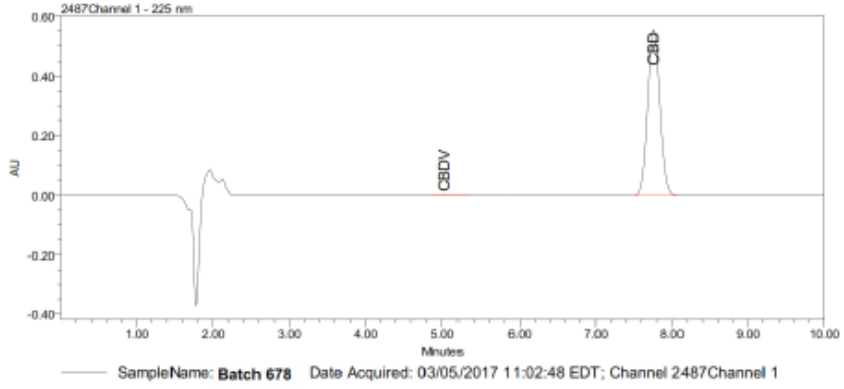


2. ANALYTICAL DATA

Parameter	Specification	Results
Appearance:	White to colored crystalline powder	Conforms
Identity:	HPLC	Conforms
Purity:	≥ 99 %	99.14 %
THC (Purity HPLC):	ND	ND
^{1}H NMR	Positive	Positive
Total impurities	≤ 1 %	0.58 %

Endoca Aps
Borupvang 3, 2750 Ballerup
Copenhagen , Denmark
https://www.endoca.com
info@endoca.com
Phone: +45898-707-00

Sample Reference:	Batch 678
Analysis Method:	100.SM.00004
Analysis ID:	81445
Date of Analysis:	2017.05.03



Column: Pro C18, S-3 µm, 12 nm YMC-Pack column (Lot No. 12294) from YMC (Kyoto, Japan), with size of 150 x 4.0 mm I.D.

Mobile Phase: solvents mixture acetonitrile:water 75:25, 0.1 % formic acid.
Flow Rate: 0.8 ml/min
Wavelength: 225 nm
Data File Name: Batch 678.pdf
Instrument: PB0023
Sample name: Batch 678

	Peak Name	Retention Time (min)	% Area	Area	Amount	Units
1	CBDV	5.030	0.17	10459	0.10	%
2	CBD	7.760	99.83	6202363	99.14	%
None						

QUALITY CONTROL ASSURANCE (DAC Monograph)

≥99% Pharma Grade Cannabidiol (CBD)

DEFINITION

≥99% Pharma grade cannabidiol (CBD) crystals are isolated from the hemp oil, which is obtained from hemp plant aerial parts by Supercritical CO_2.

CANNABIDIOL STRUCTUAL FORMULA

- • Chemical name: (1‘*R*,2‘*R*)-5‘-methyl-4-pentyl-2‘-(prop-1-en-2-yl)-1‘,2‘,3‘,4‘-tetrahydro-[1,1‘-biphenyl]- 2,6-diol.
- • Synonyms: (–)-Cannabidiol; CBD; Cannabidiol (engl.); Cannabidiol (franz.); Cannabidiolo (ital.); Cannabidiol (span.)
- • Molecular weight: 314.46 g/mol
- • Formula: C21H30O2
- • PubChem CID: 644019;
- • CAS-No.: 13956–29–1;
- • ChemSpider ID 24547

PHYSICAL AND CHEMICAL PROPERTIES

- • Appearance:

Physical state: solid in 20°C;
Colour: colourless, clear.

- • Odour: odourless;
- • Odour threshold: n.a.;
- • PH: n.a.;

- Melting point: 66-67 □C;
- Boiling point: >250 □C;
- Solubility: soluble in organic solvents. Practically not soluble in water.

HEAVY METALS
Analysis technique: atomic absorption spectrometry (AAS) after microwave digestion and ICP-
MS according to LST EN14084:2003.
*Acceptance criteria:*NMT – 0.1 ppm (CodexSTAN 193-1995, GB2762, EC No.1881/2006, FDA)
PESTICIDES
Analysis techniques according to SDP 5.4.4.Ch.179:2014 (DCh-MS), *Acceptance criteria:* NMT - 0.01 mg/kg.
MYCOTOXINS
Analysis techniques according to SDP 5.4.4.Ch.20:2013 (ELISA) and SDP 5.4.4.Ch.22:2013 (ELISA). *Acceptance criteria:* NMT – 4 ppm
MICROBIOLOGICAL TEST
Analysis techniques according to LST ISO 21527-2:2008, LST EN ISO 11290-1:2003/A1:2005, LST EN ISO 6579:2003/AC:2006, LST ISO 4832:2006. *Acceptance criteria:*
Listeria m., 1 g – ND
Yeast, 1 g – < 1.0 x 10_1
Mould, 1 g – < 1.0 x 10_1
Salmonela, 25 g – ND
E. colli, 1 g – < 1.0 x 10_1
TERPENOIDS TEST System: GC-FID
Column: RTX-5 w/Integra-Guard, 30m, 0.25mmID, 0.25µm df
Temp. Program: 40 °C to 300 °C at 13 °C/min hold 2 min
Inlet: 250°C
Detector:350 °C
Carrier gas: Hydrogen
Injection:1µL
Dissolve 10 mg of homogenous sample in 1 ml of heptane containing 0.04 % of decane as internal standard. Place the sample solution in ultrasonic bath for 5 min and then mix for 10 sec. Mix 200 µL of prepared solution with 800 µL of pure heptane and individually analysis by GC-FID under mentioned conditions. *Acceptance criteria:* oral
Linalool - NMT 0.2 mg/kg (bw/Day)
Myrcene – NMT 0.42 mg/kg (bw/Day)
-Pinene – NMT 0.31 mg/kg (bw/Day)
-Caryophylene – NMT 1 mg/kg (bw/Day)

D-Limonene – Currently Unknown for humans
CANNABINOIDS TEST
System: HPLC-UV
Column: YMC-Pack Pro C18, 150 x 4.0 mm I.D. S-3µm, 12nm
Mobile Phase: Acetonitrile : Water (80:20), 0.1% FA
Flow rate: 0.8 ml/min Wavelenght: 225 nm
Column Temp.: 30 °C
Transfer 10 mg (±.0001) of homogenously mixed hemp oil to a 2 ml microtube, add 1 ml of methanol (HPLC grade). Sonicate mixture for 2 min and mix well for 10 sec. Dilute mixture 10 times with methanol to the final concentration of 1.0 mg/ml. 20 µL of prepared solution inject into the column. *Acceptance criteria:*
oral (LD50) - currently unknown for humans. NLT – indicated in the label.
Note: Purity ≥99% (HPLC, 225 nm)
CANNABIDIOL STRUCTURE VERYFICATION TEST
System: Bruker Biospin;
Spectrum: Proton;
Spectrometry Frequency: 400MHz;
Frequency relative to TMS: solvent $CDCL_3$;
Temperature: 300 K;
Transfer 20 mg (±.0001) of homogenously mixed CBD crystals to a 5mm NMR tube, add 0.6 ml of deuterium chloroform. Sonicate mixture for 2 min and mix well for 10 sec. 20 µL of prepared solution inject into the column.
Acceptance criteria:

Note: NMR $_1$H spectrum must of CBD must matches with standard and show only pure CBD signals without any significant detectable impurities.

Equation A: The sample preparation of zeta potential and morphology analyses

Formulation	Pro-Pheroid®	CBD-pro-Pheroid®
For dilution to 4% Pheroid® **(PP-1)**	$C1V1 = C2V2$ $(4\%)(1000\,mL) = (100\%)V2$ $V2 = 0.4\,mL$ $V2 = 40\ \mu L\ in\ 960\ \mu L\ 0.1\ N\ HCL$ $(diluted\ with\ dH2O)$	$C1V1 = C2V2$ $(4\%)(1000\,mL) = (91.33\%)V2$ $V2 = 0.438\,mL$ $V2 = 43.80\ \mu L\ in\ 960\ \mu L\ 0.1\ N\ HCL$ $(diluted\ with\ dH2O)$
For dilution to 4% Pheroid® **(PP-2)**	$C1V1 = C2V2$ $(4\%)(1000\,mL) = (100\%)V2$ $V2 = 0.4\,mL$ $V2 = 40\ \mu L\ in\ 960\ \mu L\ 0.1\ N\ HCL$ $(diluted\ with\ dH2O)$	$C1V1 = C2V2$ $(4\%)(1000\,mL) = (95.53\%)V2$ $V2 = 0.419\,mL$ $V2 = 41.87\ \mu L\ in\ 960\ \mu L\ 0.1\ N\ HCL$ $(diluted\ with\ dH2O)$

Equation B: The percentage concentration decreased:

$$Decrease\ in\ conc. = Initial\ conc. - final\ conc.$$

$$\%\ Decrease\ in\ conc. = \frac{Decreased\ conc.}{Initial\ conc.}\ x\ 100$$

Table A: Average particle size distribution measured at baseline of formulation PP-1 and PP-2.

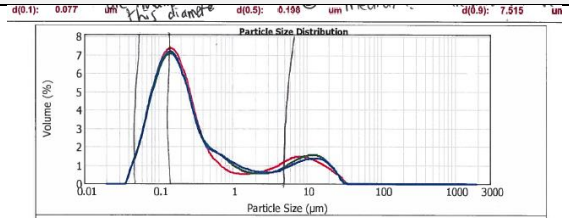
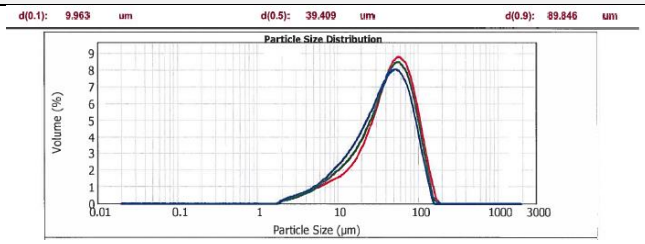
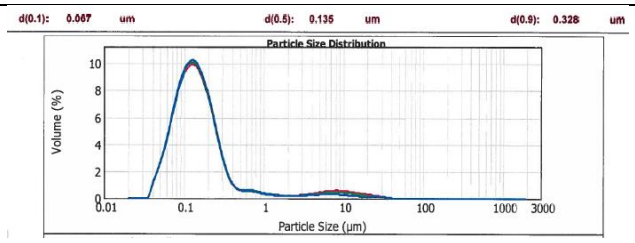
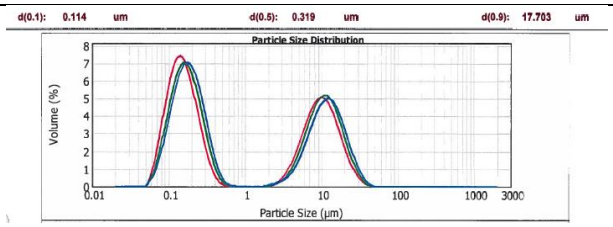
	Pro-Pheroid® (b)	**CBD-pro-Pheroid® (a)**
PP-1		
PP-2		

Table B: Average particle size distribution measured at 10 weeks of formulation PP-1a (CBD-pro-Pheroid®) and PP-1b (pro-Pheroid®).

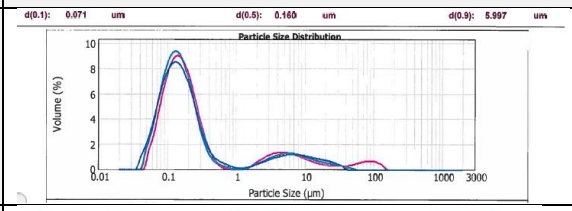
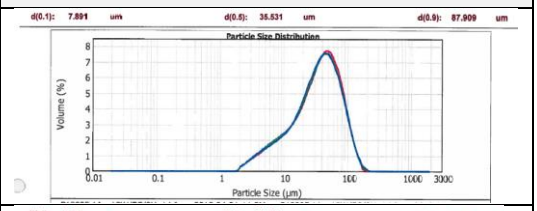
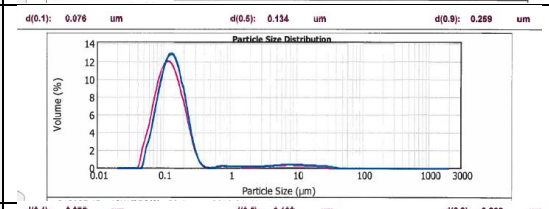
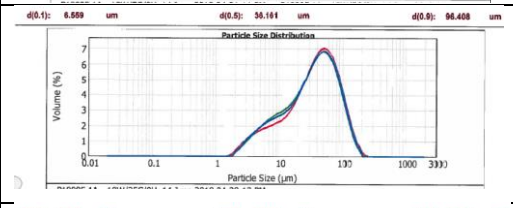
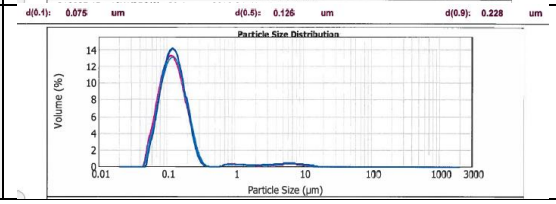
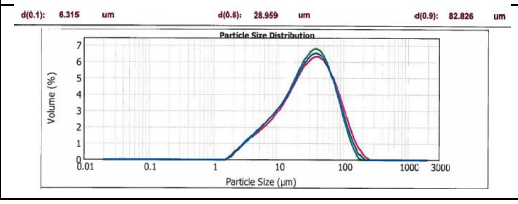
	Pro-Pheroid® (PP-1(b))	**CBD-pro-Pheroid® (PP-1 (a))**
5°C		
25°C		
40°C		

Table C: Average particle size distribution measured at 10 weeks of formulation PP-2a (CBD-pro-Pheroid®) and PP-2b (pro-Pheroid®).

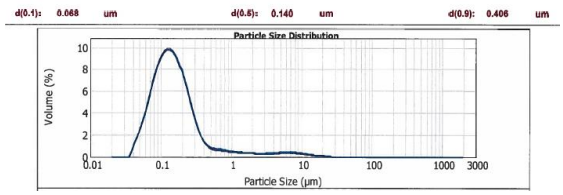
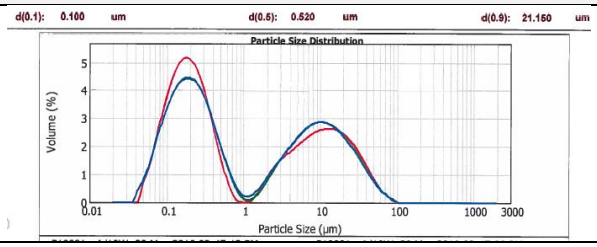
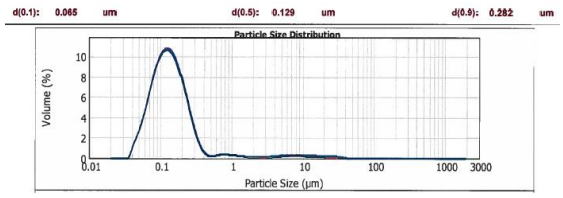
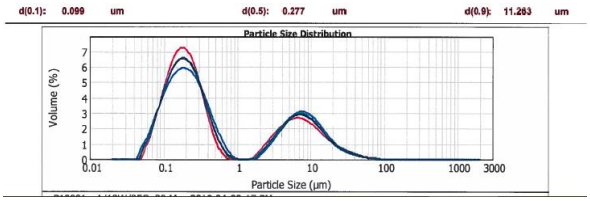
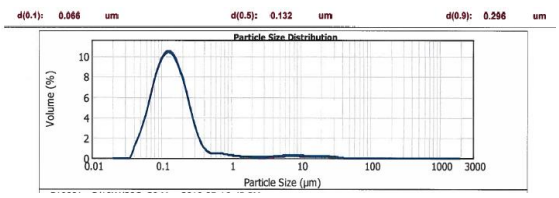
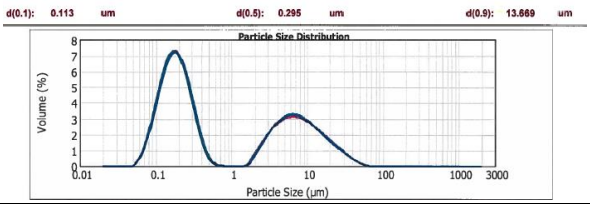
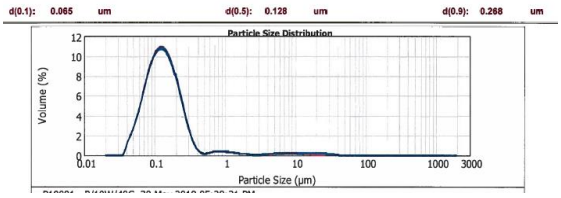
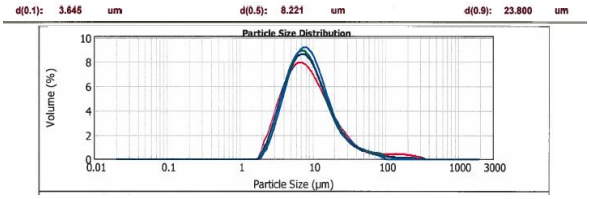
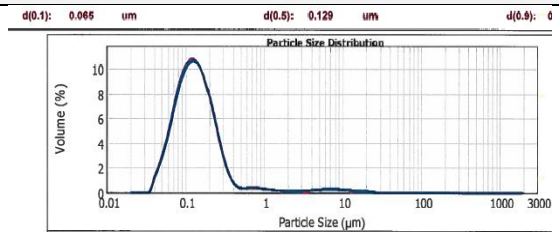
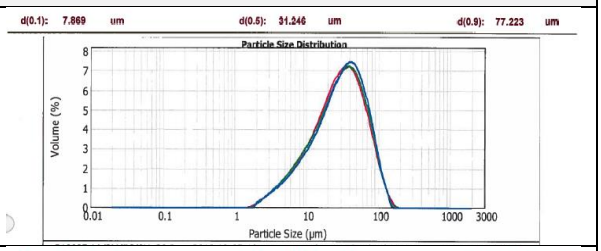
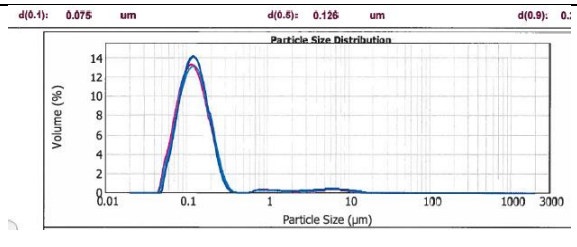
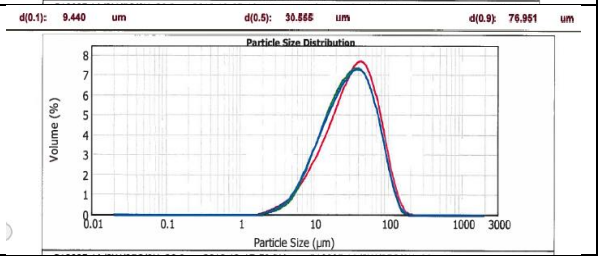
	Pro-Pheroid® (PP-2(b))	**CBD-pro-Pheroid® (PP-2 (a))**
5°C		
25°C		
30°C		
40°C		

Table D: Average particle size distribution measured at 3 months of formulation PP-1a (CBD-pro-Pheroid®) and PP-1b (pro-Pheroid®).

	Pro-Pheroid® (PP-1(b))	**CBD-pro-Pheroid® (PP-1 (a))**
5°C		
25°C		

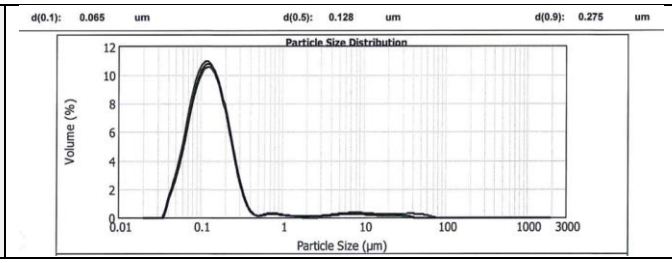
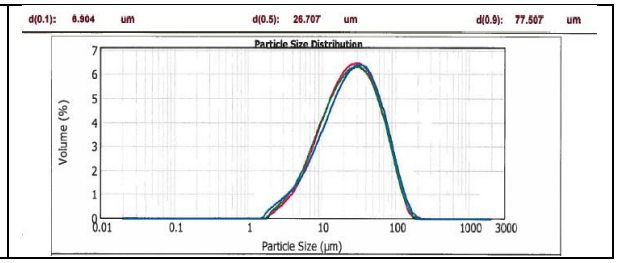
40°C		

Table E: Average particle size distribution measured at 3 months of formulation PP-2a (CBD-pro-Pheroid®) and PP-2b (pro-Pheroid®).

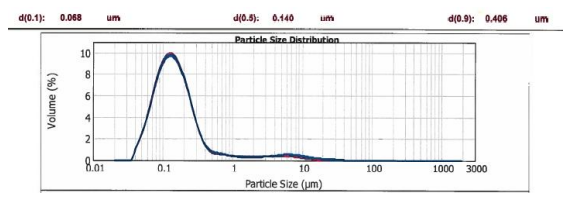
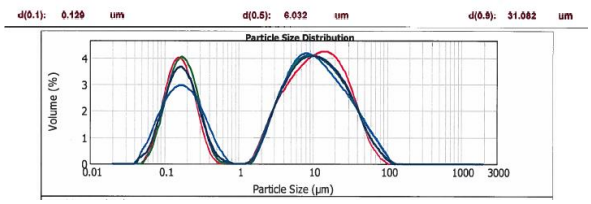
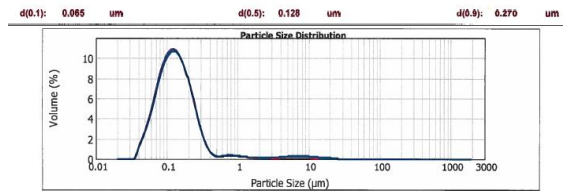
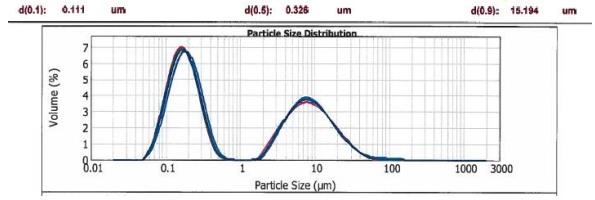
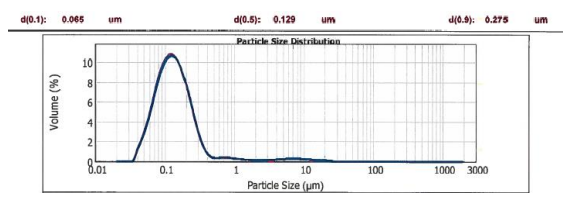
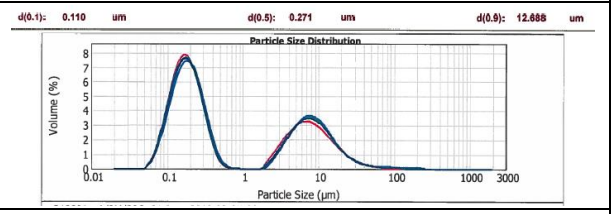
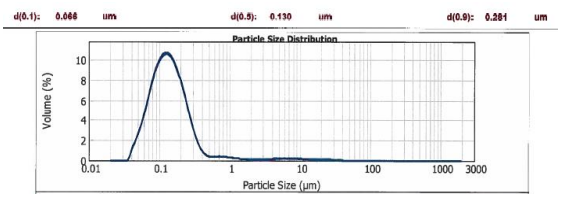
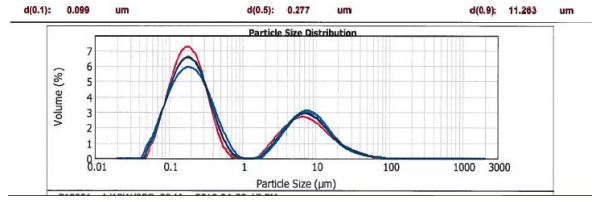
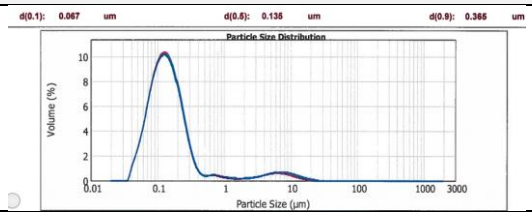
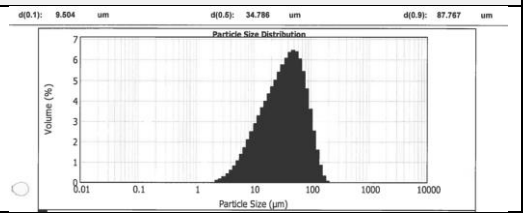
	Pro-Pheroid® (PP-2(b))	**CBD-pro-Pheroid® (PP-2 (a))**
5°C		
25°C		
30°C		
40°C		

Table F: Average particle size distribution measured at 6 months of formulation PP-1a (CBD-pro-Pheroid®) and PP-1b (pro-Pheroid®).

	Pro-Pheroid® (PP-1(b))	**CBD-pro-Pheroid® (PP-1 (a))**
5°C		

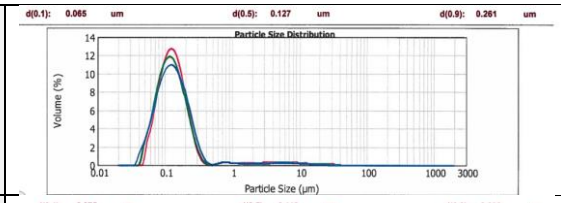
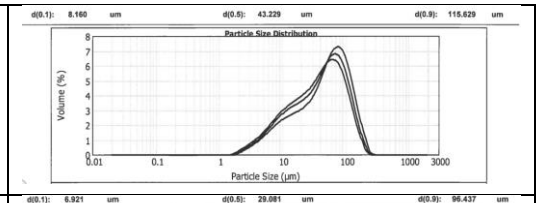
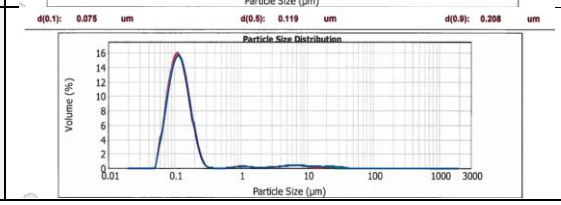
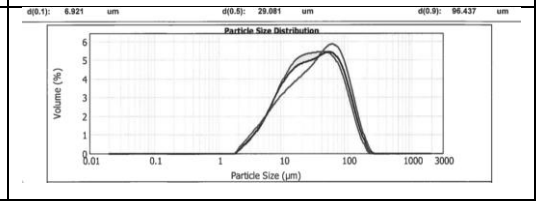
25°C		
40°C		

Table G: Average particle size distribution measured at 6 months of formulation PP-2a (CBD-pro-Pheroid®) and PP-2b (pro-Pheroid®).

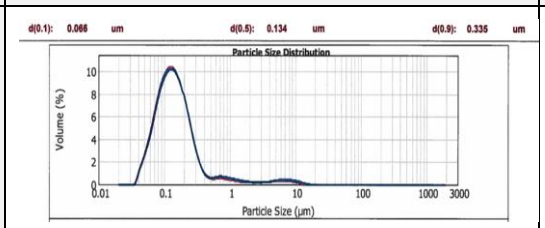
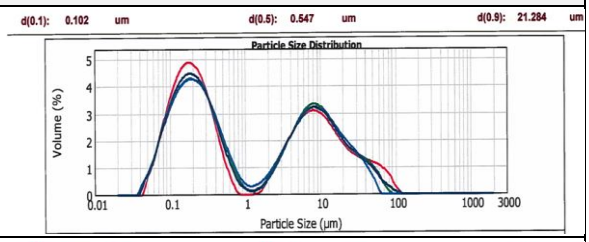
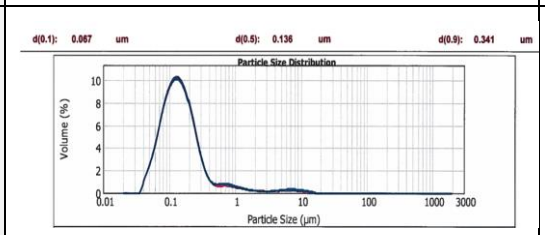
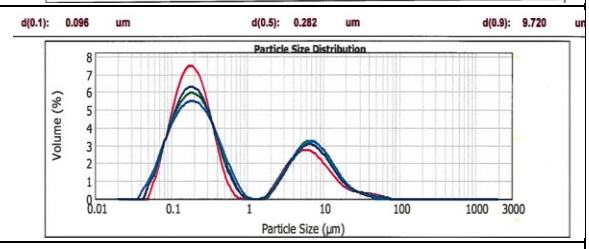
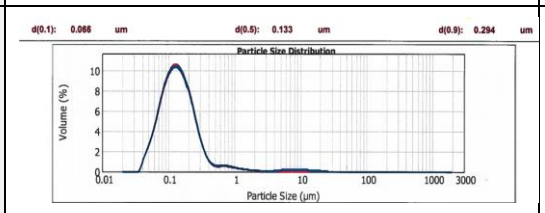
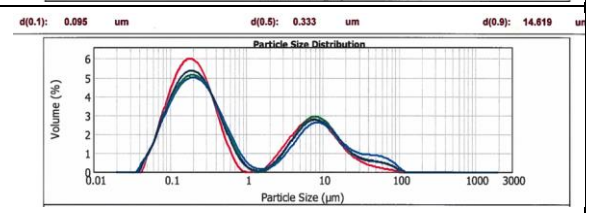
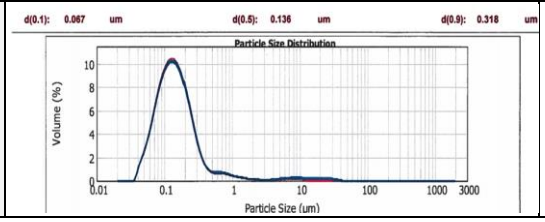
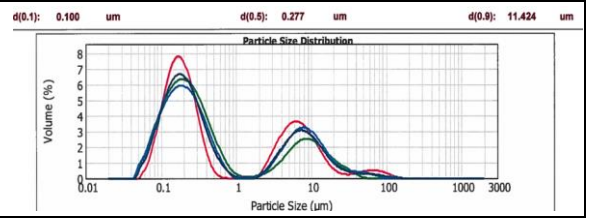
	Pro-Pheroid® (PP-2(b))	**CBD-pro-Pheroid® (PP-2 (a))**
5°C		
25°C		
30°C		
40°C		

Table A: A representation of the confocal images of PP-1 test formulation over a period of 6 months (CBD-pro-Pheroid®).*Kindly note that the brightness and contrast is not comparable and interpretations should be qualitative.

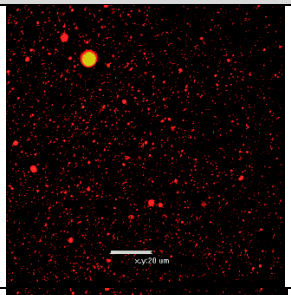
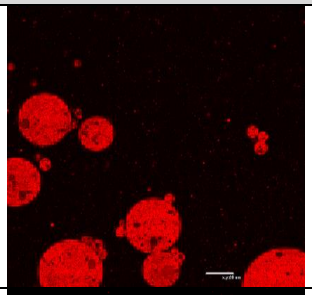
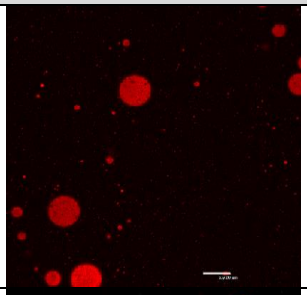
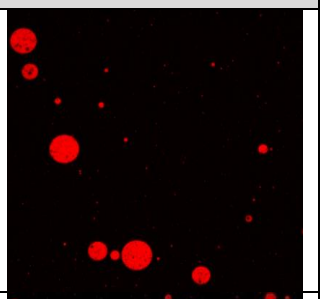
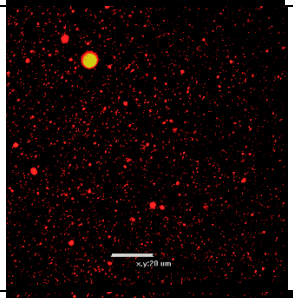
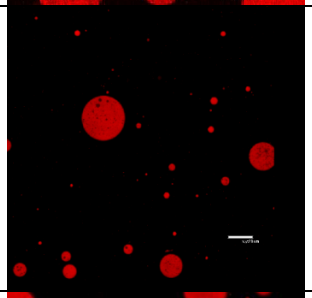
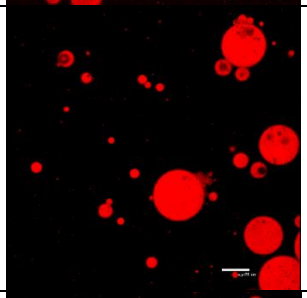
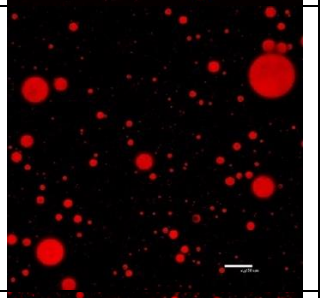
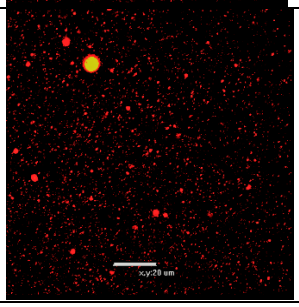
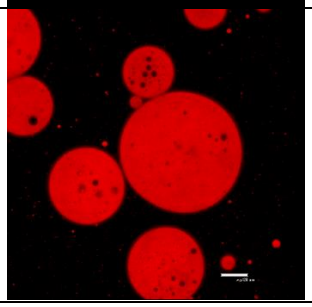
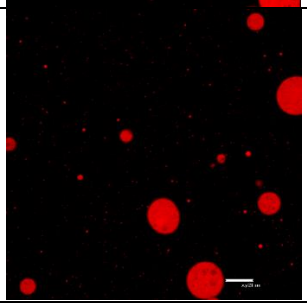
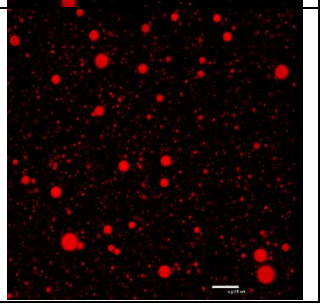
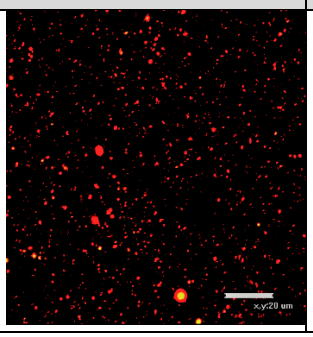
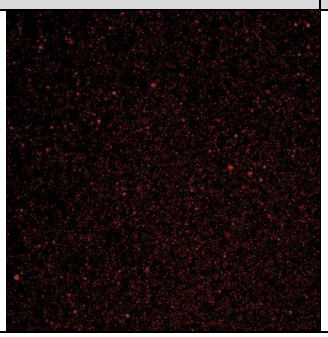
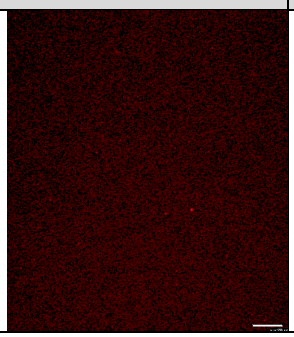
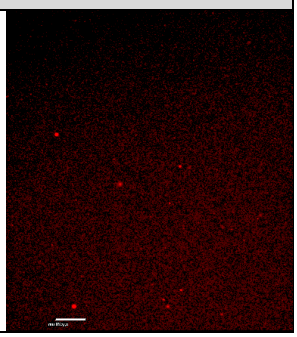
	Baseline	5°C	25°C	40°C
10 Weeks				
3 Months				
6 Months				

Table B: A representation of the confocal images of PP-1 control formulation over a period of 6 months (pro-Pheroid®).*Kindly note that the brightness and contrast is not comparable and interpretations should be qualitative.

	Baseline	5°C	25°C	40°C
10 Weeks				

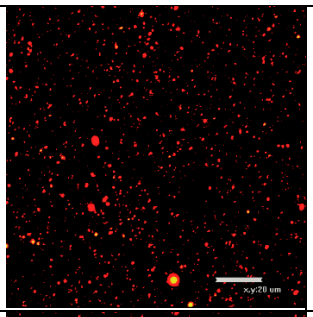
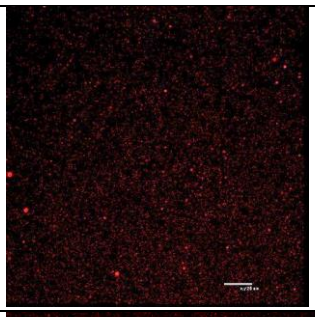
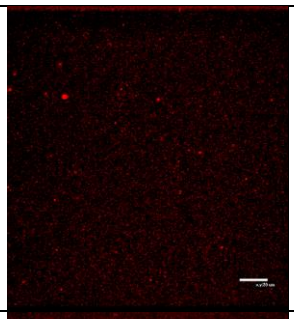
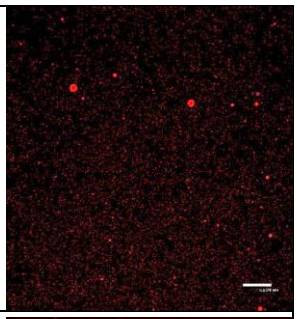
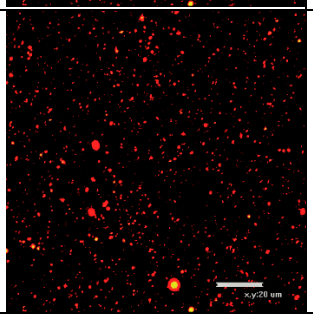
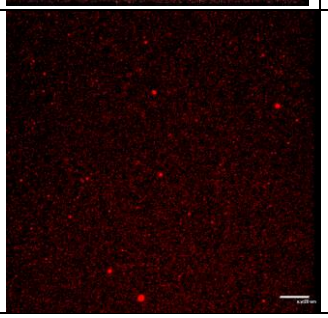
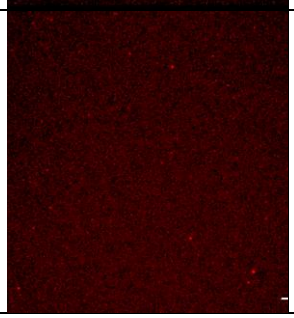
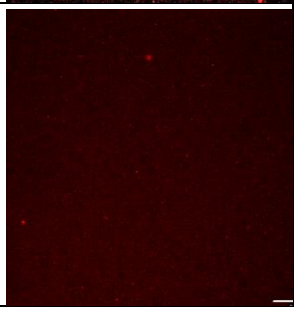
3 Months				
6 Months				

Table C: A representation of the confocal images of PP-2 test formulation over a period of 6 months (CBD- pro-Pheroid®).*Kindly note that the brightness and contrast is not comparable and interpretations should be qualitative.

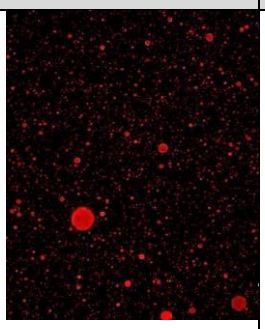
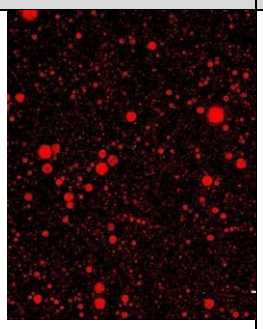
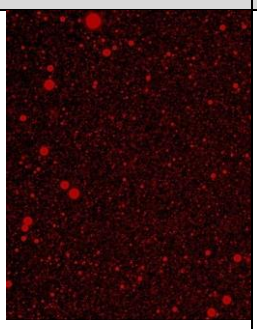
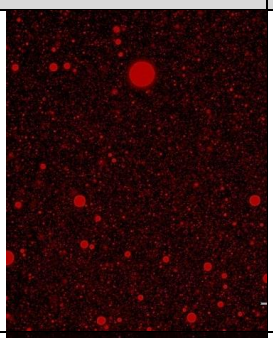
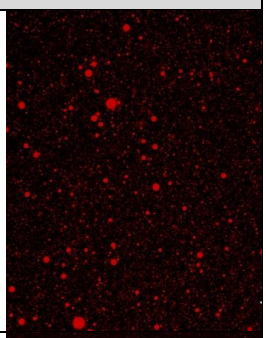
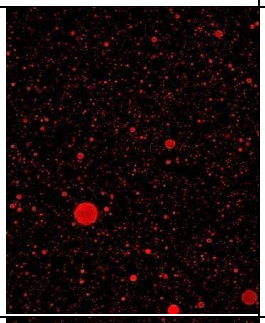
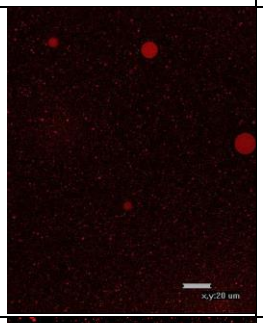
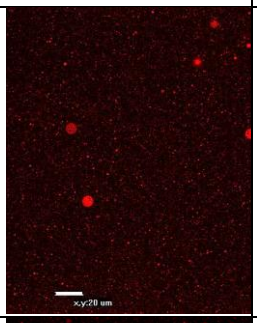
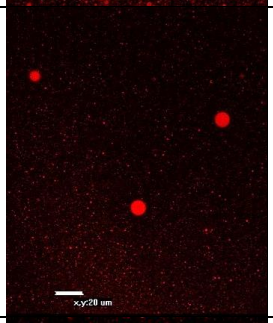
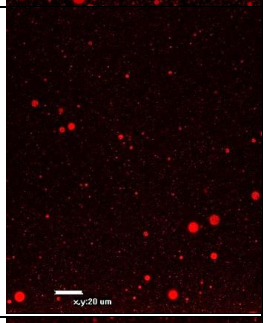
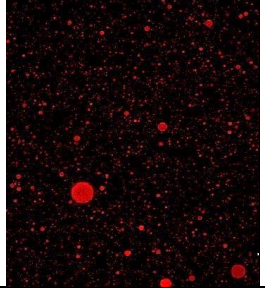
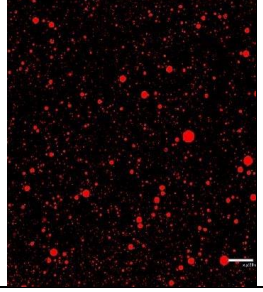
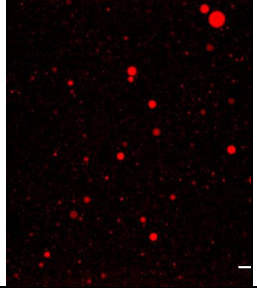
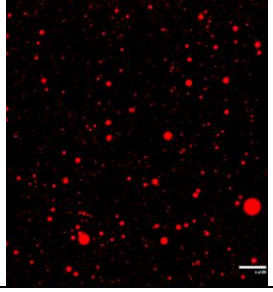
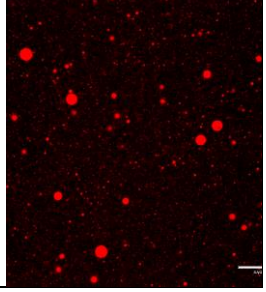
	Baseline	**5°C**	**25°C**	**30°C**	**40°C**
10 Weeks					
3 Months					
6 Months					

Table D: A representation of the confocal images of PP-2 reference formulation over a period of 6 months (pro-Pheroid®).*Kindly note that the brightness and contrast is not comparable and interpretations should be qualitative.

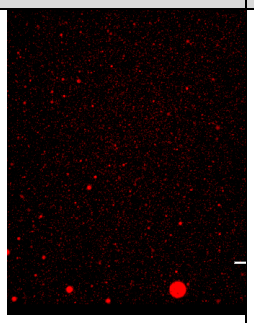
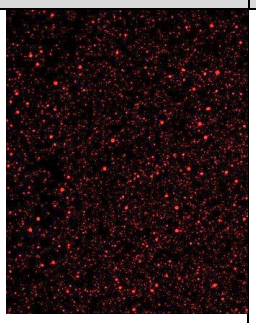
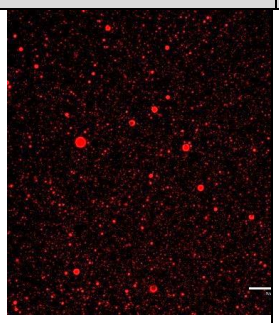
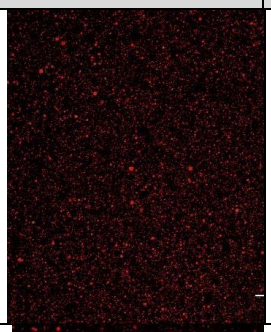
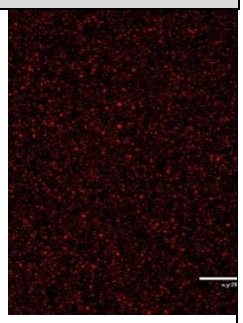
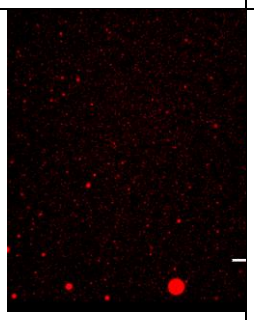
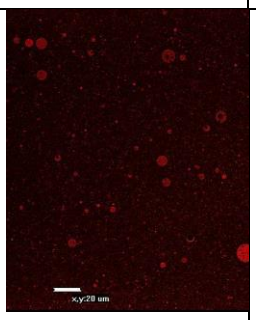
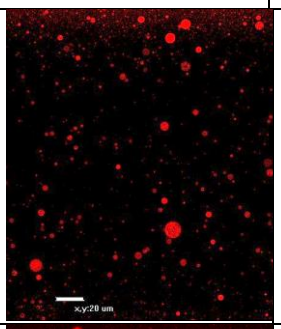
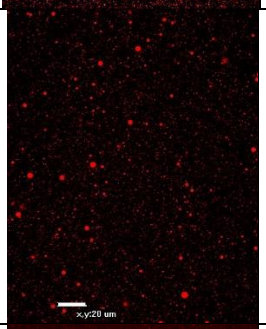
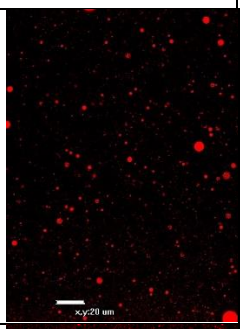
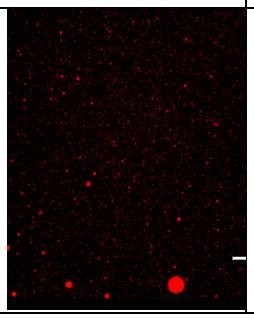
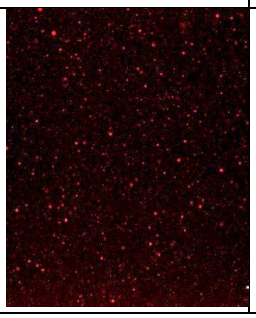
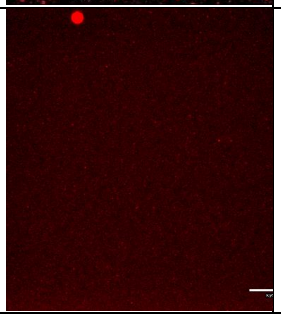
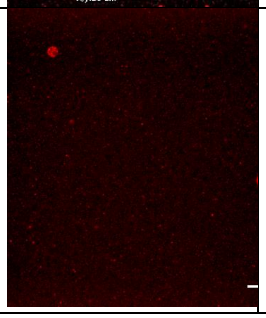
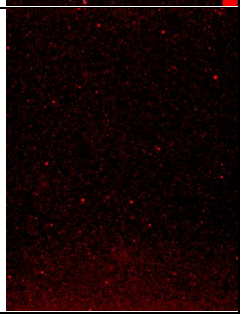
	Baseline	5°C	25°C	30°C	40°C
10 Weeks					
3 Months					
6 Months					

Table A: Average zeta potential measurements (mV) and SD of formulation PP-1 (CBD-pro-Pheroid®) for a period of 6 months.

Formulation PP-1 (CBD-pro-Pheroid®)										
	Baseline	**Week 10**			**Month 3**			**Month 6**		
		5°C	**25°C**	**40°C**	**5°C**	**25°C**	**40°C**	**5°C**	**25°C**	**40°C**
Measurement 1	-32.9	-29.53	-29.20	-20.00	-18.37	-25.43	-27.17	-24.37	-29.27	-28.20
Measurement 2	-32.9	-29.80	-27.50	-26.47	-20.87	-20.83	-25.03	-30.50	-23.20	-29.17
Measurement 3	-32.9	-29.47	-20.27	-28.17	-30.03	-23.60	-25.10	-30.30	-22.70	-26.70
Average	**-32.9**	-29.60	-25.66	-24.88	-23.09	-23.29	-25.77	-28.39	-25.06	-28.02
SD	**0**	0.18	4.74	4.31	6.14	2.32	1.22	3.48	3.66	1.24

Table B: Average zeta potential measurements (mV) and SD of formulation PP-1 (pro-Pheroid®) for a period of 6 months.

Formulation PP-1 (pro-Pheroid®)										
	Baseline	**Week 10**			**Month 3**			**Month 6**		
		5°C	**25°C**	**40°C**	**5°C**	**25°C**	**40°C**	**5°C**	**25°C**	**40°C**
Measurement 1	-29.4	-20.37	-21.57	-25.23	-22.43	-24.30	-22.67	-28.90	-24.87	-20.30
Measurement 2	-29.4	-16.67	-21.00	-26.73	-25.13	-21.90	-24.13	-30.20	-23.43	-18.25
Measurement 3	-29.4	-20.87	-20.27	-18.60	-16.07	-25.15	-22.55	-27.90	-21.20	-19.95
Average	**-29.4**	**-19.30**	**-20.95**	**-23.52**	**-21.21**	**-23.78**	**-23.12**	**-29.00**	**-23.17**	**-19.50**
SD	**0**	**2.29**	**0.65**	**4.33**	**4.65**	**1.69**	**0.88**	**1.15**	**1.85**	**1.10**

Table C: Average zeta potential measurements (mV) and SD of formulation PP-2 (CBD-pro-Pheroid®) for a period of 6 months.

Formulation PP-2 (CBD-pro-Pheroid®)													
	Baseline	**Week 10**				**Month 3**				**Month 6**			
		5°C	**25°C**	**30°C**	**40°C**	**5°C**	**25°C**	**30°C**	**40°C**	**5°C**	**25°C**	**30°C**	**40°C**
Measurement 1	-28.18	-29.13	-32.53	-32.53	-29.03	-32.40	-33.77	-33.70	-36.63	-31.20	-29.07	-28.20	-30.90
Measurement 2	-28.18	-26.37	-30.03	-30.80	-30.43	-34.27	-31.87	-35.03	-32.53	-34.23	-30.90	-29.80	-31.93
Measurement 3	-28.18	-22.73	-32.33	-32.47	-26.20	-33.80	-31.50	-33.37	-33.73	-32.03	-28.20	-30.30	-29.73
Measurement 4	-28.18	-27.27	-31.43	-27.23	-26.37	-33.73	-31.10	-31.03	-28.57	-30.83	-28.70	-34.03	-30.80
Average	**-28.18**	**-26.38**	**-31.58**	**-30.76**	**-28.01**	**-33.55**	**-32.06**	**-33.28**	**-32.87**	**-32.08**	**-29.22**	**-30.58**	**-30.84**
SD	**0.00**	**2.69**	**1.14**	**2.48**	**2.07**	**0.80**	**1.18**	**1.66**	**3.34**	**1.52**	**1.18**	**2.47**	**0.90**

Table D: Average zeta potential measurements (mV) and SD of formulation PP-2 (pro-Pheroid®) for a period of 6 months.

Formulation PP-2 (pro-Pheroid®)													
	Baseline	**Week 10**				**Month 3**				**Month 6**			
		5°C	**25°C**	**30°C**	**40°C**	**5°C**	**25°C**	**30°C**	**40°C**	**5°C**	**25°C**	**30°C**	**40°C**
Measurement 1	-28.09	-23.23	-29.03	-27.43	-28.70	-31.37	-31.17	-31.77	-30.20	-27.30	-20.30	-28.67	-29.80
Measurement 2	-28.09	-28.83	-29.73	-24.50	-25.30	-31.30	-35.37	-30.20	-30.00	-32.27	-29.10	-27.67	-30.23
Measurement 3	-28.09	-27.93	-28.87	-24.83	-25.80	-29.73	-31.73	-29.63	-27.53	-29.00	-27.20	-29.83	-29.47
Measurement 4	-28.09	-26.93	-28.33	-23.53	-27.70	-28.10	-31.80	-29.53	-29.87	-28.30	-26.57	-31.43	-28.60
Average	**-28.09**	**-26.73**	**-28.99**	**-25.08**	**-26.88**	**-30.13**	**-32.52**	**-30.28**	**-29.40**	**-29.22**	**-25.79**	**-29.40**	**-29.53**
SD	**0.00**	**2.46**	**0.58**	**1.67**	**1.60**	**1.55**	**1.92**	**1.03**	**1.25**	**2.15**	**3.82**	**1.62**	**0.69**

Figure A: The advertisement used to recruit participants for the phase 1 clinical trial.



Table A: Individual participant plasma CBD concentration(ng/mL) of both the test and the reference formulations.

CBD-pro-Pheroid® - Concentration (ng/mℓ)																	
Time (h)	**P01**	**P02**	**P03**	**P04**	**P05**	**P06**	**P07**	**P08**	**P09**	**P10**	**P11**	**P12**	**P13**	**P14**	**Mean**	**SD**	**Median**
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.50	0.27	1.46	0.32	0.00	1.38	0.00	1.24	0.91	0.66	1.06	0.24	0.00	1.71	0.00	0.66	0.62	0.50
1.00	0.74	1.06	1.10	0.54	2.20	2.06	4.95	12.46	2.88	4.44	1.60	2.12	5.67	0.12	3.00	3.21	2.06
1.50	0.94	1.41	2.13	3.94	1.98	7.25	3.55	9.55	2.95	2.84	1.06	7.57	5.40	2.82	3.81	2.66	2.84
2.00	0.59	0.73	1.23	1.42	1.03	3.76	2.57	3.81	1.20	1.79	0.89	6.36	4.12	4.79	2.45	1.81	1.79
3.00	0.37	0.47	0.68	0.76	0.77	1.29	0.92	2.85	0.69	0.87	0.58	2.24	2.94	3.44	1.35	1.05	0.87
5.00	0.24	0.28	0.31	0.48	0.41	0.48	0.48	1.66	0.30	0.45	0.36	0.99	0.87	0.96	0.59	0.40	0.48
8.00	0.15	0.26	0.23	0.22	0.36	0.32	0.22	0.93	0.27	0.22	0.20	0.36	0.44	0.36	0.32	0.19	0.27
24.00	0.11	0.14	0.15	0.12	0.13	0.14	0.19	0.23	0.14	0.13	0.13	0.19	0.20	0.15	0.15	0.04	0.14
48.00	0.00	0.14	0.12	0.11	0.00	0.12	0.14	0.16	0.12	0.12	0.11	0.14	0.13	0.12	0.11	0.05	0.12
CBD – Concentrations (ng/mℓ)																	
Time (h)	**P01**	**P02**	**P03**	**P04**	**P05**	**P06**	**P07**	**P08**	**P09**	**P10**	**P11**	**P12**	**P13**	**P14**	**Mean**	**SD**	**Median**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.13	0.18	0.00	0.00	0.13	0.00	0.00	0.04	0.07	0.00
1.5	0.15	0.00	0.00	0.17	0.00	0.14	0.23	0.14	0.42	0.00	0.00	0.16	0.00	0.00	0.10	0.13	0.07
2	0.25	0.00	0.00	0.16	0.00	0.17	0.23	0.15	0.41	0.00	0.00	0.20	0.12	0.00	0.12	0.13	0.13
3	0.14	0.00	0.00	0.16	0.00	0.23	0.28	0.13	0.22	0.00	0.00	0.17	0.15	0.13	0.11	0.10	0.13
5	0.12	0.00	0.00	0.00	0.00	0.16	0.24	0.13	0.14	0.00	0.00	0.14	0.38	0.00	0.09	0.12	0.06
8	0.14	0.00	0.00	0.00	0.00	0.14	0.20	0.12	0.12	0.00	0.00	0.11	0.77	0.00	0.11	0.20	0.06
24	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.12	0.11	0.00	0.03	0.05	0.00
48	0.12	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.00

Table B: Individual participant pharmacokinetic values of the test formulation (CBD-pro-Pheroid®).

CBD - pro- Pheroid®									
Participant	**Ke**	**AUC_inf**	**Cmax**	**Tmax**	**AUC_last**	**AUC_last/ AUC_inf**	**Cmax/A UC_inf**	**C1**	**T(1/2)**
P01	0.08	6.32	0.94	1.50	6.15	0.97	0.15	0.53	9.09
P02	0.04	12.68	1.46	0.50	10.71	0.85	0.12	0.74	15.57
P03	0.05	12.63	2.13	1.50	10.96	0.87	0.17	0.78	14.58
P04	0.06	12.26	3.94	1.50	11.16	0.91	0.32	1.03	11.96
P05	0.11	11.44	2.20	1.00	11.37	0.99	0.19	1.35	6.38
P06	0.07	18.14	7.25	1.50	17.23	0.95	0.40	1.76	10.00
P07	0.06	17.89	4.95	1.00	16.64	0.93	0.28	1.58	11.06
P08	0.08	37.30	12.46	1.00	36.30	0.97	0.33	4.47	8.34
P09	0.05	13.99	2.95	1.50	12.47	0.89	0.21	0.85	13.77
P10	0.06	14.80	4.44	1.00	13.82	0.93	0.30	1.35	10.83
P11	0.05	11.05	1.60	1.00	9.60	0.87	0.14	0.73	14.26
P12	0.07	24.48	7.57	1.50	23.49	0.96	0.31	2.66	9.28
P13	0.08	25.86	5.67	1.00	24.96	0.97	0.22	2.89	8.94
P14	0.07	20.49	4.79	2.00	19.53	0.95	0.23	1.96	9.86
Average	**0.07**	**17.09**	**4.45**	**1.25**	**16.03**	**0.93**	**0.24**	**1.62**	**10.99**
Stdev	**0.02**	**7.92**	**3.11**	**0.38**	**7.92**	**0.05**	**0.08**	**1.09**	**2.70**
Median	**0.07**	**14.39**	**4.19**	**1.25**	**13.15**	**0.94**	**0.23**	**1.35**	**10.41**

Table C: Individual participant pharmacokinetic values of the reference compound (CBD).

CBD									
Participant	**Ke**	**AUC_inf**	**Cmax**	**Tmax**	**AUC_last**	**AUC_last/ AUC_inf**	**Cmax/A UC_inf**	**C1**	**T(1/2)**
P01	0.01	20.38	0.25	2.00	3.54	0.17	0.01	0.16	100.42
P02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P04	0.03	1.48	0.17	1.50	0.52	0.35	0.11	0.18	20.01
P05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P06	0.09	2.30	0.23	3.00	2.27	0.99	0.10	0.29	7.30
P07	0.02	13.86	0.28	3.00	7.11	0.51	0.02	0.25	42.02
P08	0.03	3.07	0.15	2.00	1.91	0.62	0.05	0.15	22.82
P09	0.20	2.41	0.42	1.50	2.41	1.00	0.17	0.51	3.42
P10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P12	0.02	8.53	0.20	2.00	4.34	0.51	0.02	0.17	39.97
P13	0.12	9.22	0.77	8.00	9.17	0.99	0.08	2.04	5.70
P14	0.00	0.00	0.13	3.00	0.20	0.00	0.00	0.13	0.00
Average	**0.04**	**4.37**	**0.19**	**1.86**	**2.25**	**0.37**	**0.04**	**0.28**	**17.26**
Stdev	**0.06**	**6.32**	**0.21**	**2.13**	**2.91**	**0.40**	**0.06**	**0.53**	**28.14**
Median	**0.01**	**1.89**	**0.16**	**1.75**	**1.21**	**0.26**	**0.02**	**0.16**	**4.56**

Table A: Statistical significance when comparing the vital signs measured at baseline (t = 0) and 24 h after administration of the test formulation and the reference compound, respectively (n = 14).

Formulation	**CBD-pro-Pheroid®**		**CBD**		**Change**	
Statistic	**P-value**	**Significant**	***p*-value**	**Significant**	**P-value**	**Significant**
Systolic BP	0.749	No	0.965	No	0.796	No
Diastolic BP	0.531	No	0.553	No	0.552	No
Heart rate	0.946	No	0.323	No	0.400	No
Body temperature	0.456	No	0.959	No	0.727	No

Table B: Statistical significance when comparing the blood chemistry results measured at baseline (t = 0) and 24 h after administration of the test formulation and the reference compound.

Formulation	**CBD-pro-Pheroid®**		**CBD**		**Change**	
Statistic	**P-value**	**Significant**	***p*-value**	**Significant**	**P-value**	**Significant**
Sodium (mmol/L)	0.315	No	0.681	No	0.874	No
Potassium (mmol/L)	0.498	No	0.181	No	0.890	No
Chloride (mmol/L)	0.078	No	0.063	No	0.493	No
Bicabonate (mmol/L)	0.016	Yes	0.0001	Yes	0.985	No
Anion gap (mmol/L)	0.398	No	0.009	Yes	0.447	No
Urea (mmol/L)	0.554	No	0.721	No	0.826	No
Creatinine (µmol/L)	0.670	No	0.800	No	0.529	No
GFR	0.850	No	0.362	No	0.443	No

Table C: Statistical significance when comparing the liver enzyme levels measured, among participants, at baseline (t = 0) and 24 h after administration of the test formulation and the reference compound.

Formulation	**CBD-pro-Pheroid®**		**CBD**		**Change**	
Statistic	**P-value**	**Significant**	***p*-value**	**Significant**	**P-value**	**Significant**
Total protein (g/L)	0.067	No	0.001	Yes	0.773	No
Albumin (g/L)	0.114	No	0.001	Yes	0.850	No
Globulin (g/L)	0.317	No	0.008	Yes	0.851	No
Alb/Glob. Ratio	0.999	No	0.999	No	0.999	No
Total Bilirubin (µmol/L)	0.593	No	0.063	No	0.191	No
Conj. Bilirubin (µmol/L)	0.563	No	0.125	No	0.156	No
Unconj. Bilirubin (µmol/L)	0.610	No	0.142	No	0.233	No
ALP (IU/L)	0.685	No	0.012	Yes	0.865	No
GGT (IU/L)	0.726	No	0.014	Yes	0.482	No
ALT (IU/L)	0.717	No	0.219	No	0.213	No
AST (IU/L)	0.349	No	0.479	No	0.092	No

Table A: The mean ±(SD) electrolyte values and liver enzyme values of all the study visits and both the test and the reference formulation.

Variable	Ref	Screening	CBD			CBD-pro-Pheroid®			Close-out
n = 14			0h	24h	Change	0h	24h	Change	
Electrolytes – Mean value ± (SD)									
Sodium (mmol/L)	136-146	ND	138.64 ± (2.73)	138.93 ± (2.34)	0.29	138.00 ± (2.88)	138.71 ± (2.23)	0.71	139.15 ± (1.57)
Potassium (mmol/L)	3.5-5.1	ND	4.25 ± (0.36)	4.39 ± (0.23)	0.14	4.35 ± (0.40)	4.41 ± (0.24)	0.06	4.60 ± (0.32)
Chloride (mmol/L)	101-109	ND	106.21 ± (1.67)	105.36 ± (1.45)	-0.86	106.50 ± (1.09)	105.57 ± (1.60)	-0.93	105.08 ± (1.66)
Bicabonate (mmol/L)	21-31	ND	21.64 ± (3.48)	25.07 ± (2.53)	3.43	21.57 ± (3.18)	24.79 ± (2.15)	3.21	27.15 ± (1.21)
Anion gap (mmol/L)	03-15	ND	10.79 ± (4.89)	8.50 ± (3.61)	-2.29	9.93 ± (5.00)	8.36 ± (3.03)	-1.57	6.92 ± (1.04)
Urea (mmol/L)	2.1-7.1	ND	4.75 ± (0.94)	4.85 ± (1.10)	0.1	5.08 ± (1.40)	4.86 ± (1.06)	-0.22	5.46 ± (1.76)
Creatinine (µmol/L)	45-84	ND	78.79 ± (17.37)	78.71 ± (15.29)	-0.07	76.14 ± (20.51)	77.36 ± (17.82)	1.21	84.92 ± (18.82)
GFR	> 90	ND	106.79 ± (12.88)	108.36 ± (11.43)	1.57	108.86 ± (15.48)	108.93 ± (14.93)	0.07	98.92 ± (15.49)
Liver tests – Mean value ± (SD)									
Total protein (g/L)	66-83	74.57 ± (4.72)	66.46 ± (3.78)	70.36 ± (4.88)	3.9	66.57 ± (4.42)	69.36 ± (4.16)	2.79	72.42 ± (4.08)
Albumin (g/L)	35-52	44.50 ± (3.88)	41.21 ± (2.67)	44.00 ± (2.94)	2.79	41.50 ± (3.08)	43.36 ± (2.50)	1.86	44.50 ± (1.68)
Globulin (g/L)	21-35	30.07 ± (5.58)	24.92 ± (2.90)	26.36 ± (3.23)	1.43	25.07 ± (3.08)	26.00 ± (3.21)	0.93	27.92 ± (3.15)
Alb/Glob. Ratio	0.9-2.7	1.54 ± (0.35)	1.71 ± (0.25)	1.71 ± (0.22)	0	1.69 ± (0.27)	1.68 ± (0.25)	-0.01	1.63 ± (0.18)
Total Bilirubin (µmol/L)	05-21	9.92 ± (5.44)	9.64 ± (2.47)	8.43 ± (2.10)	-1.21	8.43 ± (3.39)	9.07 ± (3.12)	0.64	10.46 ± (1.61)
Conj. Bilirubin (µmol/L)	< 3.4	2.08 ± (1.19)	2.14 ± (0.86)	1.79 ± (0.70)	-0.36	1.64 ± (0.63)	1.86 ± (0.77)	0.21	2.00 ± (0.43)
Unconj. Bilirubin (µmol/L)	02-17	7.85 ± (4.30)	7.50 ± (1.79)	6.64 ± (1.55)	-0.86	6.79 ± (2.91)	7.21 ± (2.49)	0.43	8.42 ± (1.56)
ALP (IU/L)	30-120	66.79 ± (17.80)	64.00 ± (19.38)	67.36 ± (20.35)	3.36	64.14 ± (20.68)	68.86 ± (22.64)	4.71	66.92 ± (17.87)
GGT (IU/L)	< 38	19.50 ± (9.04)	17.00 ± (6.47)	18.64 ± (8.16)	1.64	18.29 ± (7.43)	18.79 ± (7.63)	0.5	21.92 ± (9.78)
ALT (IU/L)	< 35	20.07 ± (11.65)	19.00 ± (11.22)	21.50 ± (17.48)	2.5	21.79 ± (15.98)	21.36 ± (16.59)	-0.43	26.46 ± (23.34)
AST (IU/L)	< 35	24.86 ± (9.80)	22.71 ± (12.38)	23.23 ± (13.74)	0.52	25.50 ± (14.06)	23.07 ± (13.52)	-2.43	23.00 ± (7.30)

1. Scope

The purpose of this narrative document is to describe the events of the clinical study titled 'A clinical investigation into the bioavailability of a *peroral* cannabinoid-Pheroid® formulation' in detail.

2. Capsulation

For each study arm, capsules were weighed individually by hand, after which CBD were added to 16 capsules and pro-Pheroid® to 8 of those capsules. There were 8 CBD control capsules and 8 CBD-pro-Pheroid® capsules for each study arm. One capsule for each participant and 1 additional capsule of both the CBD and the CBD-pro-Pheroid® formulations were capsulated as an extra. The capsules were formulated 4 days prior to each study arm as to ensure the drug was in the same condition when given to the participants. The pro-Pheroid® and CBD used were from the same batch. The drug was encapsulated in a red opaque capsule as demonstrated in figure 1.

a) *b)*

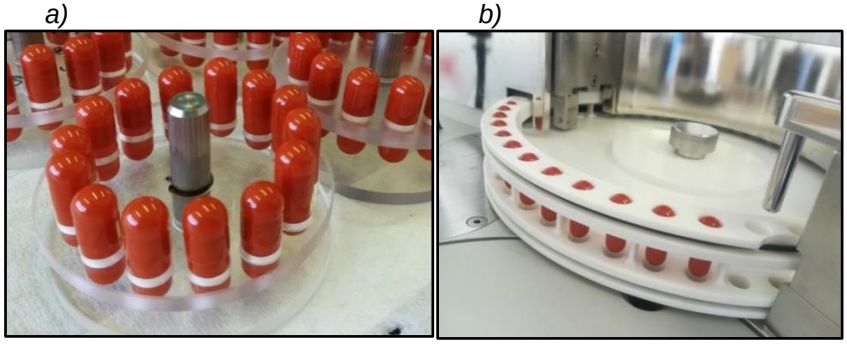


Figure 1: (a) representation of the capsules used (b) Capsules were sealed by utilizing the of CFS 1200 capsulation machine

Each capsule was individually placed in an amber glass container to ensure that the blinding was maintained. The capsule was checked by two individuals to ensure the correct capsule was given to the correct participant each study arm. The information contained on the label included the participant number, batch number, date of manufacturing, storage conditions and study arm date.

a) *b)*



Figure 2: (a) representation of the containers used (b) Containers with labels used during the trial.

3. Advertisement and recruitment

Advertisement was done according to protocol. Posters were put up around Potchefstroom, excluding campus. Sites included Bult, Protea Boekwinkel, Spar, Cachetpark Pharmacy, MooiRivier Mall etc. Advertisement was also done on the Potchefstroom Facebook group every three days and this was where most of the participants were recruited. Flyers were also handed to potential volunteers at the Bult area and MooiRivier Mall. Participants showed interest by either email or Whatsapp. It is advisable to create an alternative email address for the PCDDP for future studies. This will ensure that more than one person can correspond with the participants.

One-on-one information sessions were held. It was done in English, Afrikaans and Setswana – depending on the volunteer. The sessions were held by trained persons. They were not remunerated for their time as the protocol stated that they had to be independent from the study. Refreshments were provided to volunteers who joined a session and they received the information in the form of a hard copy power-point presentation, the Informed Consent Form (ICF), a questionnaire containing questions of the session and a list of what they should remember during each study visit. All questions regarding the study were answered. It is advisable to rather have group sessions. Even though one-on-one sessions accommodated the volunteers, this was time consuming and could be improved upon by organising a few larger sessions.

4. Screening of participants

Volunteers who were interested in participating in the study signed the ICF in the presence of both the researcher and the independent person. The time signed was also noted. The questionnaire was reviewed to ensure the participant fully understood what the study entailed. The participants completed the Medical History Form and asked questions if they did not understand a question. A photocopy of the volunteer's identification document was made. A date and time was scheduled for the volunteer to be screened. The screening included the physical examination. Blood pressure and heart rate were measured in triplicate. The physical examination also included height, weight, temperature and blood glucose measurements. A pregnancy test was given to each female volunteer. A blood sample was also collected by direct venepuncture

in a 3 mL gel blood collection tube. The tube was placed on a cooling pad and was sent to Pathcare Laboratories to determine

the volunteer's liver function. Each volunteer received a screening number to ensure anonymity. Pathcare Laboratory results were received within 24-hours of screening. The Principal Clinical Investigator (PCI), Dr. Emile Kotze, reviewed the results and determined the participants eligible for participation in the study based on his medical professional knowledge.

22 Participants were screened during the study and 14 were enrolled. Volunteers were given the opportunity to discuss the results with the PCI. Health status reports containing screening results were provided to each volunteer.

5. Training

Each member involved in the clinical trial had to complete training. These included the clinical staff members, the responsible pharmacist, the blood preparation technicians and the members who explained the study to potential participants. All members were trained on the protocol, how to correctly complete clinical trial documents and their responsibility during the study. Each member had to complete the Training Log according to GCP. During this study there was one Training Log for each day. It is advisable for future studies to compile a log for each responsibility rather than each training day.

6. Planning

The clinical trial took place at MooiMed Private Hospital in Potchefstroom, Ward 2. The ward consisted of 7 rooms, where the male and female participants were separated. Each room could contain 2 or 3 participants and also had an en-suite bathroom and access to the outside courtyard (figure 3). The participants were allowed to leave their rooms but were not permitted to leave the clinic until the last blood sample collection for the day was completed.

a) *b)* *c)* *d)*

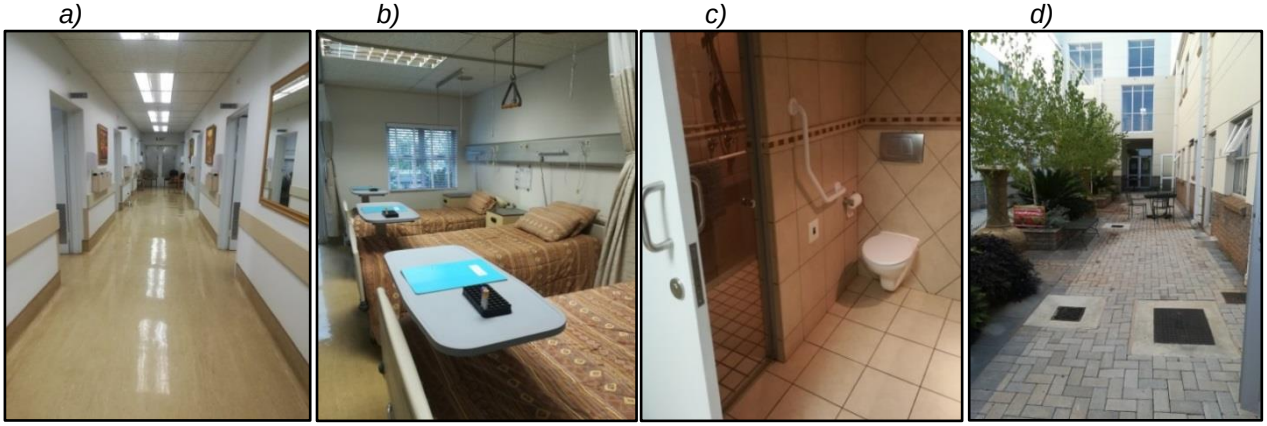


Figure 3: (a) an image of the overall ward consisting of 7 rooms (b) the room containing a bed for each participant (c) each room had a bathroom containing a shower (d) the courtyard

Each room had an assigned nurse. Entertainment was provided in the form of television as well as board games. The participants were encouraged to bring their own work or entertainment for the course of the study arm. The clinical trial coordinator reminded each participant the specific products to avoid 24 hours prior to the study arm and the time each participant should arrive. The products which should have been avoided included tobacco, alcohol, drugs and caffeine-contained products. The equipment and consumables were prepared beforehand and set up the previous night.

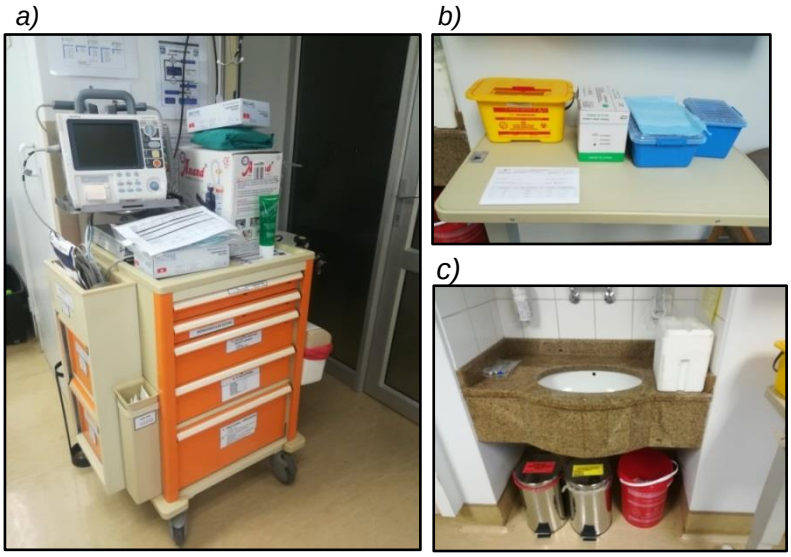


Figure 4: (a) emergency equipment with a defibrillator (b) the equipment and sharps container (c) the waste bins, biological waste bins and the polystyrene container

Each room in the ward contained a set of necessary equipment and consumables as seen in figure 3c. There was also a sharps container and a biological waste bin in each room. Extra batteries and pens were provided to the responsible nurse. There was also a polystyrene container in each room as collected blood samples had to go on ice immediately after collection according to protocol. According to GCP guidelines there was also an emergency trolley with a defibrillator.

7. Study arms

7.1 Arrival of members

Clinical staff arrived at 06:00 and prepared their equipment. Each staff member signed the Delegation Log, Contact List and Signature List. Participants started arriving at 08:00. A Bidvest car was rented to transport participants who did not have transport or who preferred to be picked up. Each participant received a randomised participant number. This was randomly assigned prior to the start of the study arm. Each participant had a different arrival time. This was done to stagger the participants. Each room had one participant arrive every half hour. This provided enough time for the participant to settle in and for the responsible nurse to take baseline values. The baseline

values were directly entered into the CRF. The CRF also contained questions regarding the participant and had to be completed by the nurse before baseline samples were collected. The CRF contained only documents needed for the indicated study arm as to minimise confusion and wrong entries into the CRF. The CRF was marked with the participant number as to provide each participant with confidentiality.

7.2 Baseline

Following the arrival of all the participants Dr. Kotze evaluated each participant's baseline values and determined whether a participant could be included in the study. The baseline evaluation included the physical examination. Blood pressure and heart rate were measured in triplicate. The physical examination also included height, weight, temperature and blood glucose measurements. A pregnancy test was given to each female volunteer.

He then signed the CRF and inserted either a pink (20G) or a green (18G) Jelco® cannula into the arm vein of the participant. It was avoided to insert the Jelco® into the hand as this could be a probable cause of haemolysis. The Jelco® was connected to a J-Loop (large) and secured in place by using Tegaderm plasters as seen in figure 6a. The Jelco® was then flushed using a saline and heparin mixture. The fasted baseline sample was taken by discarding the first 2 mL of blood and then withdrawing 2 mL blood slowly into the 5 mL syringe. This was done to minimise the blood haemolysis. If a participant experienced discomfort due to the Jelco® and requested it to be removed, a new Jelco® could be inserted.



Figure 6: Representation of the Jelco® inserted into each participant

Two baseline samples were collected. One (collected in a 3 mL gel blood collection tube) was sent to Pathcare Laboratories and the other (collected in a 3 mL EDTA blood collection tube) was used to determine the baseline CBD level. It was critical that the samples do not undergo haemolysis as multiple safety tests, stated in the protocol, could not be completed by Pathcare due to presence of haemolysis in collected samples. To decrease the pre-analytical variability of the blood chemistry results, haemolysis was prevented during this study by including the following precautions:

- By slow withdrawal of the blood.

- By discarding the first 1 mL of each collected blood sample.
- By flushing the J-loop with saline, following each blood sample withdrawal.
- By performing blood sample preparation immediately (within 10 min of sample collection).

Figure 7 represents different levels of haemolysis. After each sample collection the time collected and the label on the sample was written in the CRF. The blood sample was placed in the polystyrene container where it was picked up by a runner, the time of collection was noted and the sample was taken to the blood preparation station.

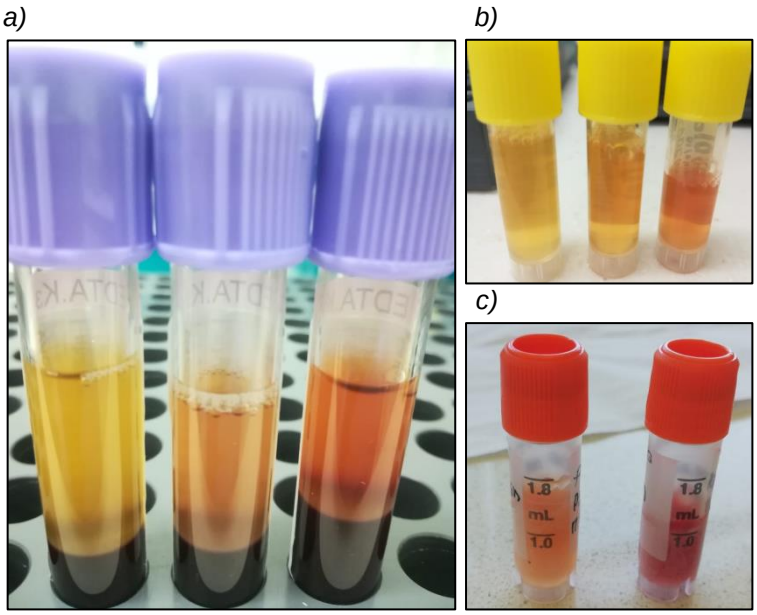


Figure 7: (a) Increasing levels of haemolysis in the EDTA collection tubes (b) increasing levels of haemolysis in cryovials (c) unaccepted levels of haemolysis (not used during analysis)

7.3 Dosing

Participants were allowed to drink water before dosing but were prohibited to drink or eat anything else as the interaction between CBD and food is unclear in literature. After the baseline sample was collected, the responsible pharmacist checked that each participant swallowed the correct capsule from the correct container (figure 8). This was also checked by the researcher. Both the pharmacist and the researcher signed the CRF.



Figure 8: Labelled capsule container and blood collection tubes

Each participant was provided with a water bottle to be taken with the capsule. The capsule was administered by the responsible pharmacist and checked. The time that the capsule was taken was written above each bed to keep track of the collection times. There were no participants who struggled with drinking the capsule and there were no instances where a second capsule was given to a participant. The participants were not allowed to eat directly after drinking the capsule and were provided food after the 30 min blood sample collection. Participants received similar food, taking allergies and food preference into account. Juice and decaffeinated products were also provided to the participants. Adverse events were documented and listed in the Adverse Event Log.

7.4 Blood Sample Collections

Blood sample collections were taken at baseline, 30 min, 1h, 1h 30 min, 2h, 3h, 5h, 8h, 24h and 48h post capsule administration. The 3 mL EDTA blood collection tubes and the 2 mL cryovial tubes were labelled prior to the study arm to optimise time usage. The staff members were reminded to check the tube labelling as they proceeded to collect the blood. Dr. Emile was on call during the blood sample collections should anything happen. After the blood samples were collected and directly put on ice, the runner received and checked the samples. The samples were taken to the blood preparation station. There the Medical technologist and an assistant signed receipt of samples. Samples were centrifuged for 10 minutes according to the protocol at 4˚C and 1500 rpm. After the samples were centrifuged, the plasma was separated into cryovial tubes by utilizing a pipette and sterilised tips. The tubes were placed on dry ice to maintain the integrity of the sample as CBD is unstable in heat. Different cryovial tubes were used during study arm 1 and 2.



Figure 9: (a) Dry ice was used to freeze samples on site (b) organised labelled cryovial tubes

The participants were taken home after the 8 h sample collection and were prohibited to drink alcohol and caffeinated products until the completion of the study arm.

7.5 24-Hour blood sample collection

The participants returned to MooiMed Hospital for the last blood sample collection. A physical examination was performed and the fasted blood sample was collected by direct venepuncture into two collection tubes. One (collected in a 3 mL gel blood collection tube) was sent to Pathcare Laboratories and the other (collected in a 3 mL EDTA blood collection tube) was used to determine the CBD levels at 24 hours after drug administration. Adverse events were documented and listed in the Adverse Events Log. The participants received refreshments and dinner before leaving the hospital.



Figure 10: The setup of tubes during the 24-hour blood collection

7.6 48-Hour blood sample collection

All participants returned to the Health Clinic at the North-West University to complete the 48 Hour blood sample collection. The 48-hour blood sample was to determine the CBD concentration 48 hours after administration of the CBD formulation. All participants completed the 48-hour blood sample collections and all participants complied with the study protocol.

8. Study Close-out

Study close-out was done after one month, based on each participant's schedule. One participant failed to attend a close-out session. The visit included a physical examination, together with a blood sample withdrawal to determine liver enzyme and blood chemistry values. Participants also completed a questionnaire regarding their experience and perception of the investigational product.

9. Conclusion

All study documents were filed and archived appropriately as indicated in the protocol. The clinical trial and all study visits were successfully executed. This study provides a reference for future clinical trials, either general trials, or trials investigating a CBD-preparation.

END OF REPORT