universität wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

Solubility enhancement of a synthetic cannabinoid by different cyclodextrins

verfasst von / submitted by

Silvia Paunova

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Magistra der Pharmazie (Mag.pharm.)

Wien/ Vienna, 2016

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 449
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Diplomstudium Pharmazie
Betreut von / Supervisor:	O. Univ.-Prof. Mag. Dr. Helmut Viernstein

ACKNOWLEDGEMENTS

First of all I would like to thank O. Univ.-Prof. Mag. Dr. Helmut Viernstein for offering me the opportunity to participate in this very interesting diploma thesis.

I would also like to express gratitude to Ao. Univ.-Prof. Dr. Peter Wolschann for his full support, expert guidance, incredible patience and encouragement throughout my research and writing process.

My deepest, most sincere appreciation goes out to my parents, Dipl. oec. Konstantin Paunov and Dipl. jur. Zaneta Paunova, and my brother, soon to be Mag. pharm. Daniel Paunov, who are always there for me.
I hope that with these words I can express how truly grateful and thankful I am for having you in my life, for your kind words and encouragement to believe in myself no matter what happens.

Last but not least I would like to thank everyone at the Department of pharmaceutical technology who helped me during the research.

LIST OF CONTENTS

1. ABBREVIATIONS LIST

Δ⁹-tetrahydrocannabinol (Δ⁹--THC),

Δ^8-tetrahydrocannabinol (Δ^8-THC),

Δ⁹-tetrahydrocannabivarin (Δ⁹-THCV),

Cannabidiol (CBD),

2-arachidonylglycerol (2-AG),

Anandamid (AEA),

Fatty acid binding proteins (FABPs),

Endocannabinoid system (ECS),

Gamma-aminobutyric acid (GABA),

Polymerase chain reaction (PCR),

Transient receptor potential vanilloid 1 (TRPV1),

Nerve growth factor (NGF),

Arachidonic acid (AA),

Fatty acid amide hydrolase (FAAH),

Monoacylglycerol lipase (MAGL),

Matrix metalloproteinase-3 (MMP-3),

Receptor activator of NF-κB ligand (RANKL),

Chemokine (C-C motif) ligand2 (CCL2),

Fibroblast-like synoviocytes (FLS),

Macrophage colony-stimulated factor (M-CSF),

Glucose 6-phosphate isomerase (GPI),

Triosephosphate isomerase (TPI),

N-oleoylethanolamine (OEA),

N-palmitoylethanolamine (PEA),

Dietary-induced obese (DIO),

Pyruvate kinase isozyme (PKM1),

Lactate dehydrogenase (LDHa), Glyoxalase-1 (Glo1),

Tricarboxylic acid (TCA) also known as citric acid cycle,

Dihydrolipoamide dehydrogenase (DLD),

Nuclear peroxisome proliferator-activated receptors (PPARg)

2. AIM OF STUDY

Due to the low aqueous solubility of Δ^9-THC, the biologically active cannabinoid of Cannabis sativa, it is obvious that nabilone, which is chemically similar to Δ^9-THC, suffers from the same problem and represents an obstacle for the development of more active administration forms.

The purpose of this study was to examine the effect of cyclodextrins (CDs), precisely randomly methylated β-cyclodextrin (RAMEB) and hydroxypropyl β-cyclodextrin (HP-β-CD), on the aqueous solubility of nabilone and further on the stability of the nabilone:CD complexes.

Also it is demonstrated that in an ethanolic/ aqueous solution the cosolvent as solubilizing agent increases the solubility. However, the fact that it contains ethanol is resulting in frequent irritation of the administration site.

The resulting nabilone-CD complex was studied via UV and lyophilization-studies.

Filtration and stability tests where used to investigate the time depending decrease of concentration leading to the assumption that a different stoichiometric complex is formed after some time. Based on the obtained results, 1:1 and 1:2 complexes are enhancing the solubility whereas the stoichiometric complex leading to a lower concentration is probably a 2:1 complex.

3. INTRODUCTION

3.1 Cannabinoids

Phytocannabinoids or plant cannabinoids are a set of compounds found in Cannabis sativa plant and the physiological effects have been used for both leisure and medicinal purposes since ancient times thus have gathered immense interest for pharmaceutical development nowadays.

Δ^9-Tetrahydrocannabinol is the main psychoactive cannabinoid and has attracted a great attention as a new class of antiemetic and appetite stimulation, both associated with chemotherapy HIV/AIDS, agent by mimicking endogenous substances- the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) which are the main modulators of the important immunmodulator system and lipid signaling – the ECS .

Cannabinoid based medicines targeting the ECS holds notable therapeutic promise in a variety of pathological situations including obesity, stroke, diabetic complications, glaucoma, inflammation, neuropathic pain, epilepsy, spasms, hypertension, cancer, psychosis, addiction, and neurodegenerative diseases including multiple sclerosis, Parkinson's and Alzheimer's disease. (Pacher et al., 2013)

These uses include wasting–syndrome in AIDS patients, antiemetic (in patients receiving cancer chemotherapy), anti-anxiety, neuroprotective, analgetic (especially in cancer pain) and anti-inflammatory effects. (Husni et al., 2015)

During the last years evidences obtained that cannabinoids can reduce tumor growth in animal models and exert palliative effects on cancer-associated symptoms. (Velasco et al., 2016)

Other studies showed that THC is a bronchodilator, an antipruritic agent in cholestatic jaundice, a neuroprotective antioxidant and has 20 times the anti-inflammatory power of aspirin and twice that of hydrocortisone. (Russo, 2011)

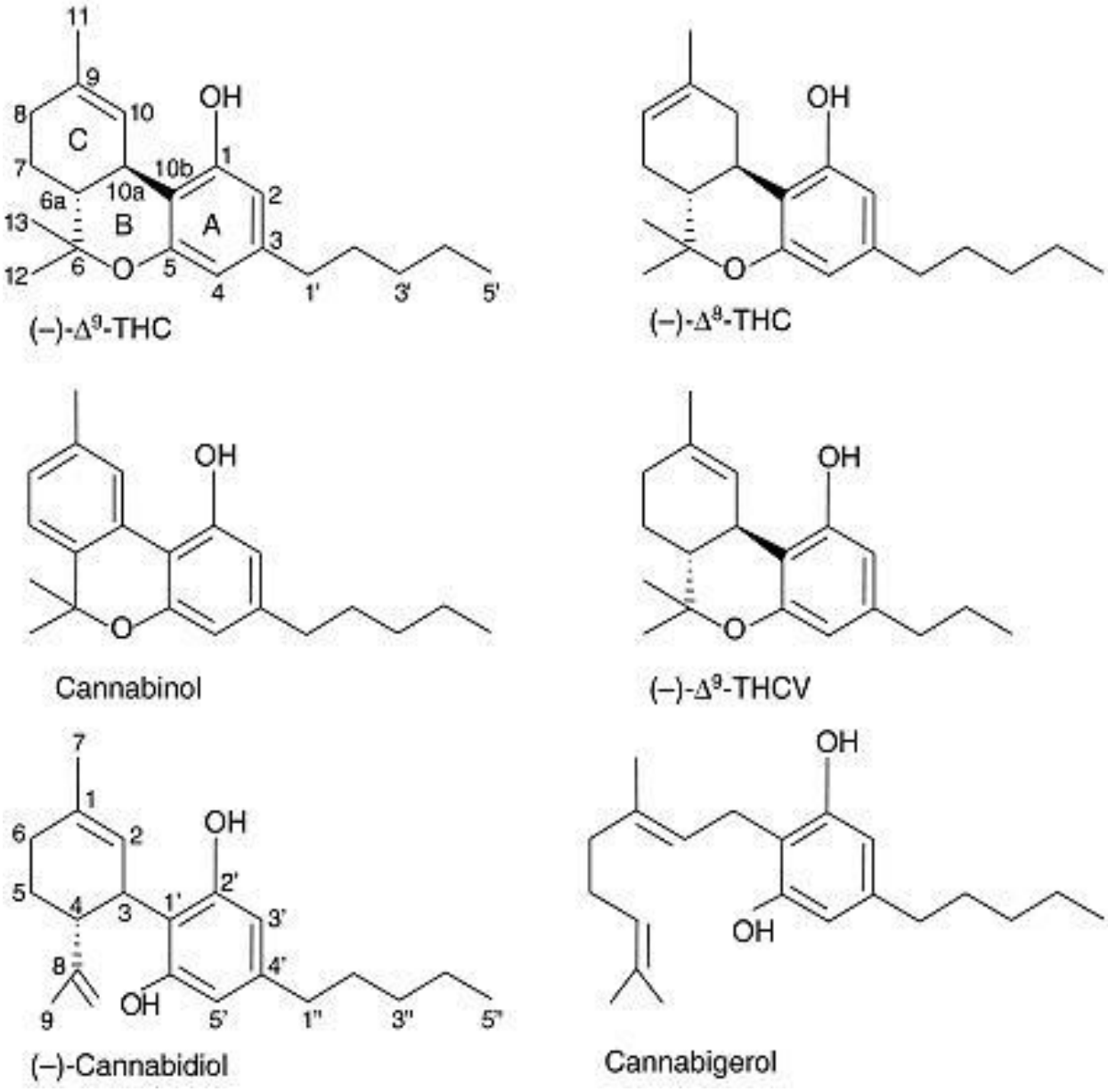
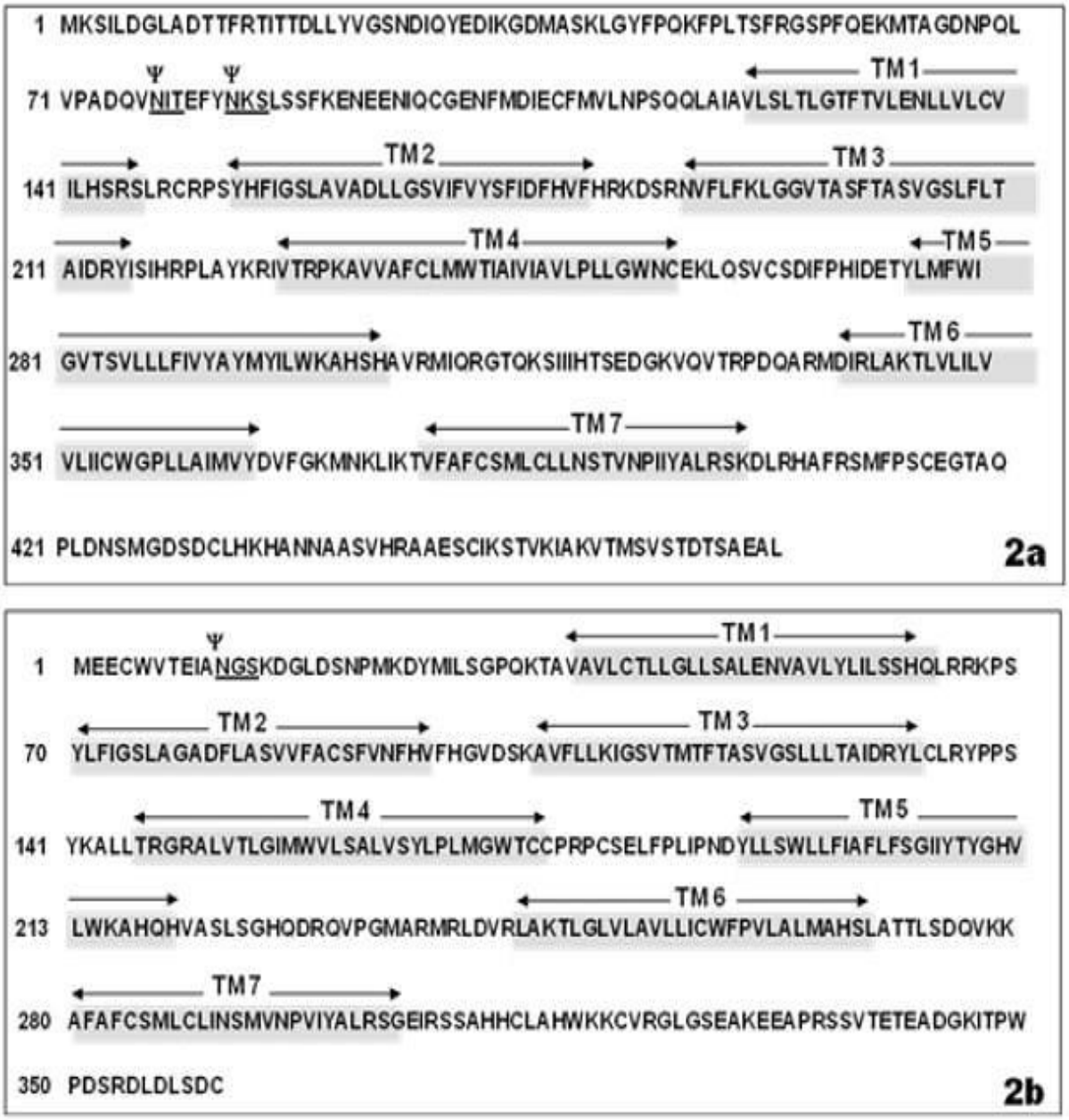


Figure 1: Structures of the phytocannabinoids. Δ^{9}-tetrahydrocannabinol (Δ^{9}-THC), Δ^{8}-tetrahydrocannabinol (Δ^{8}-THC), cannabinol, Δ^{9}-tetrahydrocannabivarin (Δ^{9}-THCV), cannabidiol (CBD) and cannabigerol.

(Pertwee, 2008)

3.1.1 Receptors

The mechanism can be explained through the function of the cannabinoid receptor system. Cannabinoid receptors belong to the G-protein-coupled receptors (GPCR) family, which consists of the classical CB1 and CB2 and signal via Gαi/o proteins.



Figures 2a and 2b. Human cannabinoid receptor 1 (CB1). Amino acid sequence of the full-length human CB1 receptor. The putative asparagine-linked glycosylation sites are shown as Ψ. The seven trans-membrane domains are highlighted and noted TM 1 through TM 7. The Genebank accession numbers are NM_016083, NM_001840 and NP_057167.2. Panel 2b. Human cannabinoid receptor 2 (CB2). Amino acid sequence of the full-length human CB2 receptor. The putative asparagine-linked glycosylation site is shown as Ψ. The seven trans-membrane domains are highlighted and noted TM 1 through TM 7. The Genebank accession numbers are NM_001841 and NP_001832. (Cabral G. A., 2009)

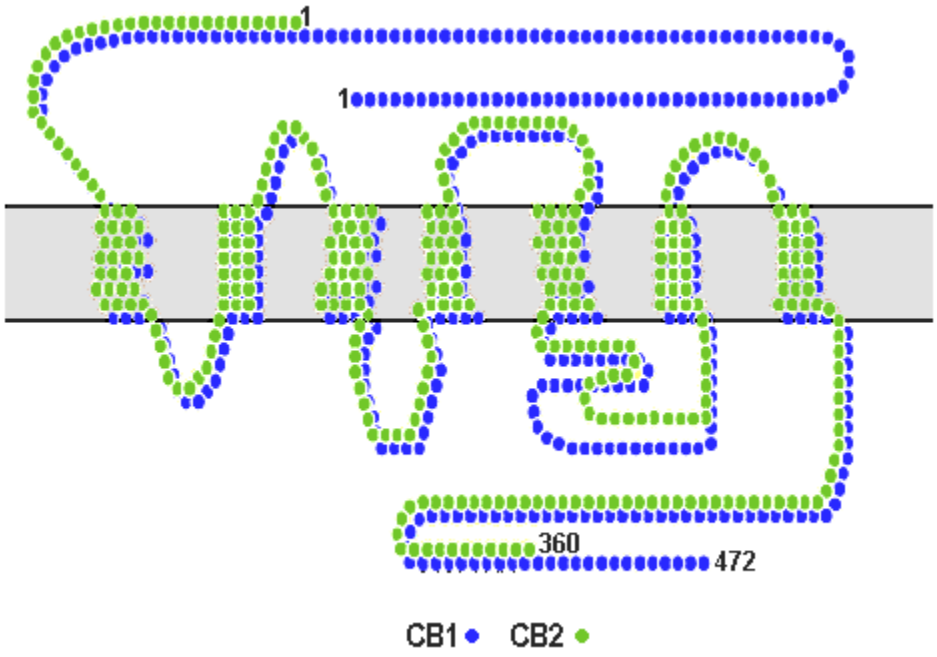


Figure 3: Cannabinoid receptor structure. (www.pterodattilo.com)

The CB1 receptor, first cloned in 1990 by Matsuda et al, is encoded by the CNR1 gene and is widely expressed and mainly located in neuronal tissue such as cerebellum, hippocampal formation and basal ganglia, regions of the brain that are involved in the coordination and initiation of movement, (Reza et al., 2015) as well as in the spinal cord, digestive tract, liver cells, pituitary gland, lungs thyroid gland, fat cells, kidneys, adrenal gland, muscle cells and male and female reproductive organs. (Husni et al., 2015)

Further, the CB1 receptor can mediate inhibition of ongoing release of different excitatory and inhibitory transmitters, like noradrenaline, acetylcholine, 5-hydroxytryptamine (5-HT), glutamate, dopamine, gamma-aminobutyric acid (GABA), D-aspartate and cholecystokinin. (Pertwee, 2008)

The amino acid sequence for CB1 showed 472 amino acids, one less than other mammalian species. (Figure 2a)

The gene for the CB2 receptor, cloned from HL60 cells 1993 by Munro et al. and from rat by Griffin et al. in 2000**,** is CNR2 and was shown to encode for a 360 amino acid long, 7 transmembrane G-protein receptor (Figure 2b) that is comparable to CB1. Furthermore it was found to have an extracellular, glycosylated N-terminus and an intracellular C-terminus.

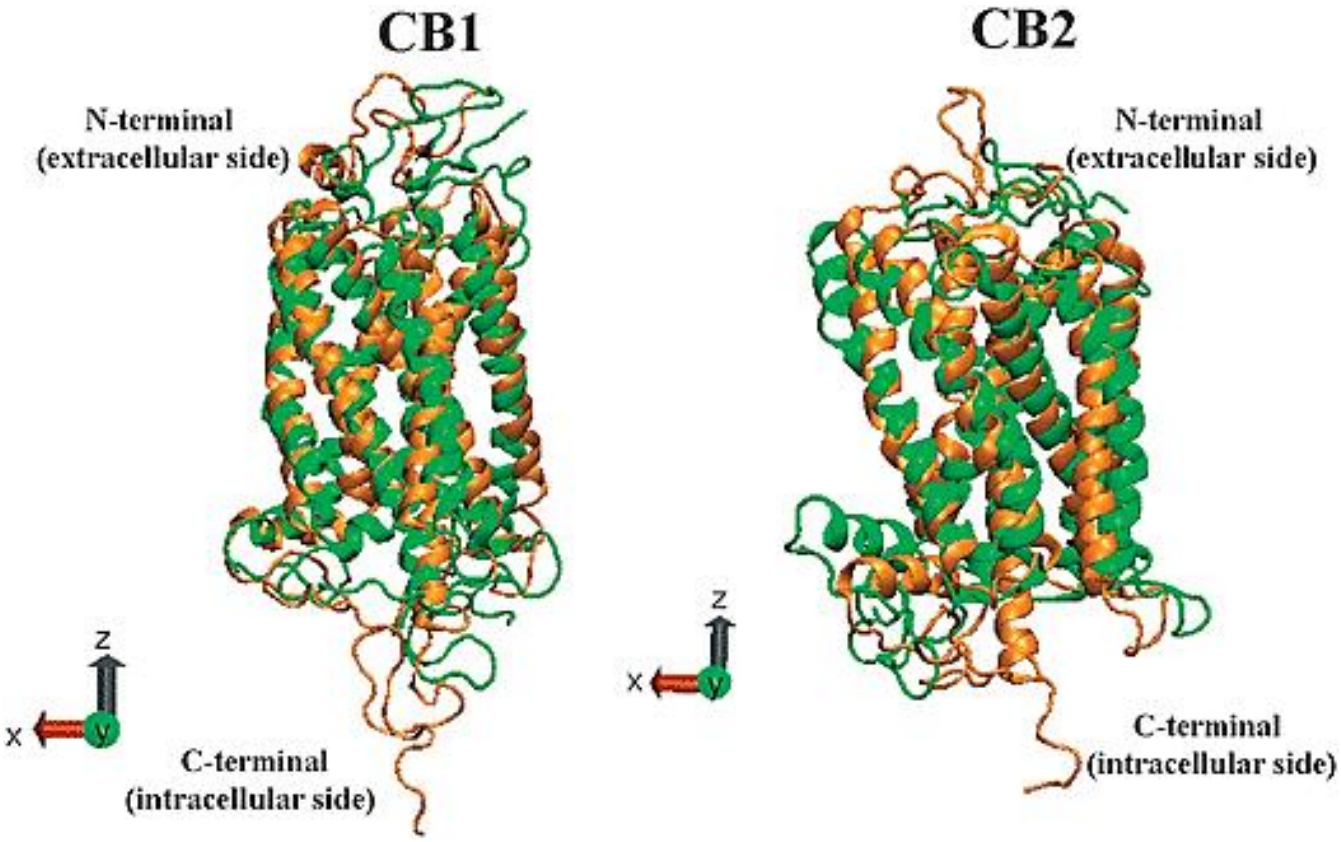


Figure 4: cannabinoid receptor 1 / cannabinoid receptor 2
(Ramos et al., 2011)

CB2 receptors are widely expressed throughout the gastrointestinal system but their main circulation is mostly in cells and tissues of the immune system including the B lymphocytes, thymus, T lymphocytes, tonsils, monocytes, macrophages and natural killer (NK) cells which is another distinctive feature of CB2 in comparison to CB1. (Cabral et al., 2009)

The CB1 and CB2 receptors have approximately 44% similarity of their amino acid sequences. (Munro et al., 1993)

The most powerful approach to find out which receptor is important for mediating the effects of cannabinoids in animals has been the development of mice in which either the CB1 gene or the CB2 gene has been deleted.
The study showed that CB1- knockout mice have been unresponsive to cannabinoids in a standard set of behavioral assays and the immunomodulatory effects of cannabinoids in CB2–knockout mice were absent whilst behavioral effects mediated by CB1 are normal. (Elphick et al., 2001)

The activation of the CB1 and CB2 receptors produce many of their effects by enhancing K^+ channels and depressing various voltage gated Ca^{2+} channels. Deadwyler et al. (1995) showed that activation of presynaptic cannabinoid receptor by cannabinoids enhance activation of A-type potassium currents due to inhibition of adenylyl cyclase, leading to decreased phosphorylation of the channel by cAMP-dependent protein kinase A (PKA). Thus, causes shortening the duration of action potentials which may influence the release of neurotransmitter
Another signaling pathway, that was demonstrated by Mackie and Hille in 1992, is the inhibition of the N-type voltage-gated calcium channels in neuronal cells through direct Gi/o protein interaction, leading to reduction of important synaptically evoked Ca^{2+} signals. (Elphick et al., 2001)

3.1.2 Synthesis and metabolism of endocannabinoids

The role of the major ECBs, such as arachidonoyl-ethanolamide (anandamide, AEA) and 2- arachidonoyl glycerol (2-AG), as retrograde messengers that suppress both excitatory and inhibitory transmission are well-established.

AEA is well spread in the brain and shows high distribution in the striatum, hippocampus, thalamus, brainstem and to a lesser extent in the cerebral cortex and cerebellum (Buczynski et al., 2010) whereas lower concentrations are found in human serum, plasma, and cerebrospinal fluid.

2-AG is detected in both the brain and periphery, albeit its concentration is 150 times higher in brain compared to that of AEA. Higher 2-AG levels are found in rat hippocampus, brainstem, striatum, and medulla (Bisogno et al.,1999). (More et al., 2015)

The synthesis of endocannabinoids arises by hydrolysis of membrane lipid precursors.

Anandamide is developed by cleavage of its precursor N-arachidonoyl phosphatidylethanolamine via phospholipase D, whereas 2-AG is created by cleavage of its precursor diacylglycerol via diacylglycerol lipase. Anandamide (Willoughby et al., 1997) and 2-AG (Jarai et al., 2000) are both degraded into arachidonic acid (AA). Fatty acid amide hydrolase (FAAH) is the main hydrolyzing enzyme for anandamide (Cravatt et al., 1996), whereas the main hydrolyzing enzyme for 2-AG is monoglyceride lipase (MGL) (Dinh et al., 2002). (Walter et al., 2004)

New Cannabinoid receptor GPR55 & TRPV

In 2007 Ryberg et al. investigated the expression pattern of GPR55 in a panel of mouse tissues using quantitative PCR and suggested that the G-Protein coupled receptor GPR55 may be a novel cannabinoid receptor because it is targeted by a number of cannabinoids. Surprisingly it is activated as potently and with greater efficacy than the CB1 receptor by the main psychoactive compound of cannabis (Δ^9-THC). Furthermore, anandamide, the predominant circulating endocannabinoid, activated GPR55 with a potency equivalent to that activating CB1 and CB2 receptors. (Pertwee, 2007)

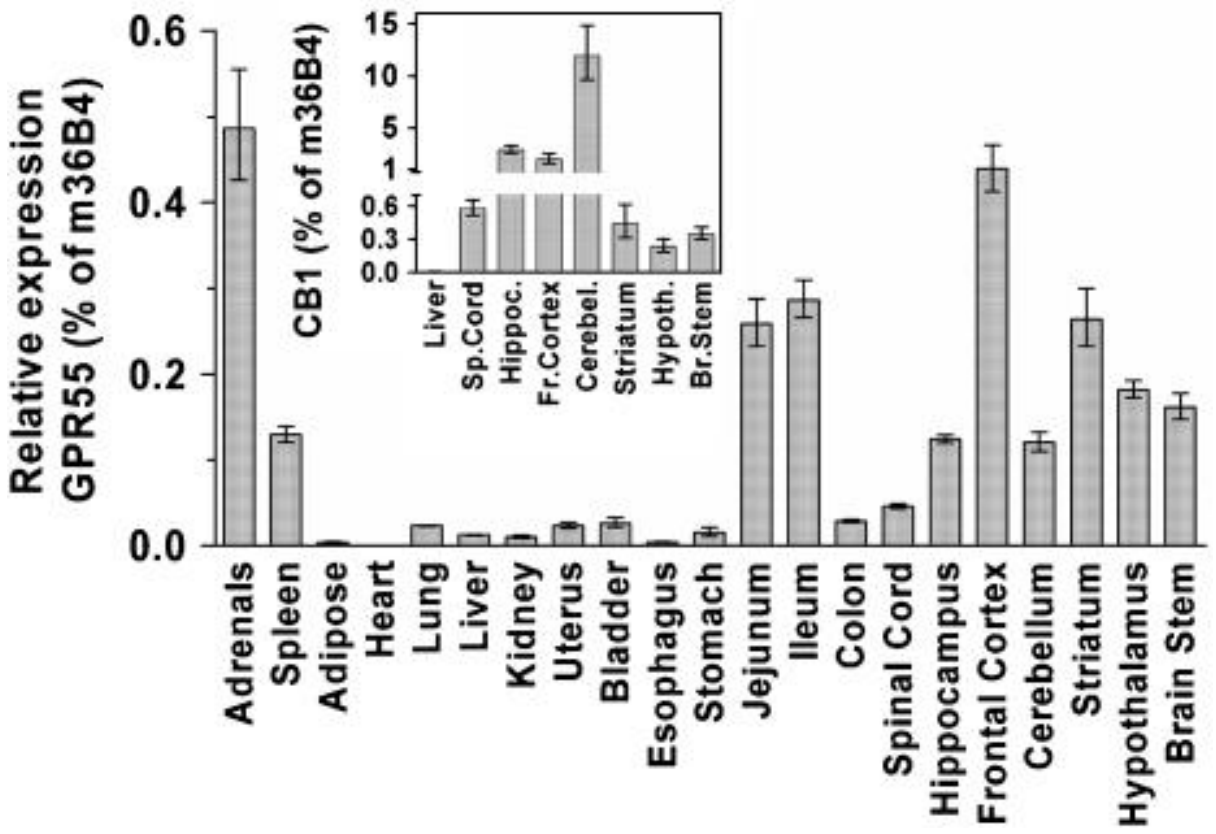


Figure5: mRNA expression levels of GPR55 and CB1 receptors in mouse tissues measured by quantitative PCR relative to m36B4. Tissues were dissected from C57BL/6 female mice. Samples from different mice were processed individually in all subsequent steps; RNA preparation, cDNA synthesis and quantitative PCR. Data are mean values7s.e.m. using tissues from eight (GPR55) or four mice (CB1) and presented as per cent of the ubiquitously and homo- genously expressed ribosomal protein 36B4. (Ryberg et al., 2007)

It is known that the CB1 and CB2 signaling promote primarily via Gαi/o proteins and adenylate cyclase to activate multiple different downstream cascades. However, it is established that the signal transduction pathway of GPR55 differs from that of CB1 and CB2 because it has been proved that GPR55 only couples to Gα12 and 13 proteins, thereby activating ras homologue gene family member A (RhoA) and Rho-associated protein kinase (ROCK). The activation of RhoA and ROCK evokes the phospholipase C (PLC) pathway to raise intracellular Ca^{2+}. Furthermore, it activates small GTPase proteins rhoA, Rac and cdc42, and turns on extracellular regulated kinase (ERK) phosphorylation. (Yang et al., 2015)

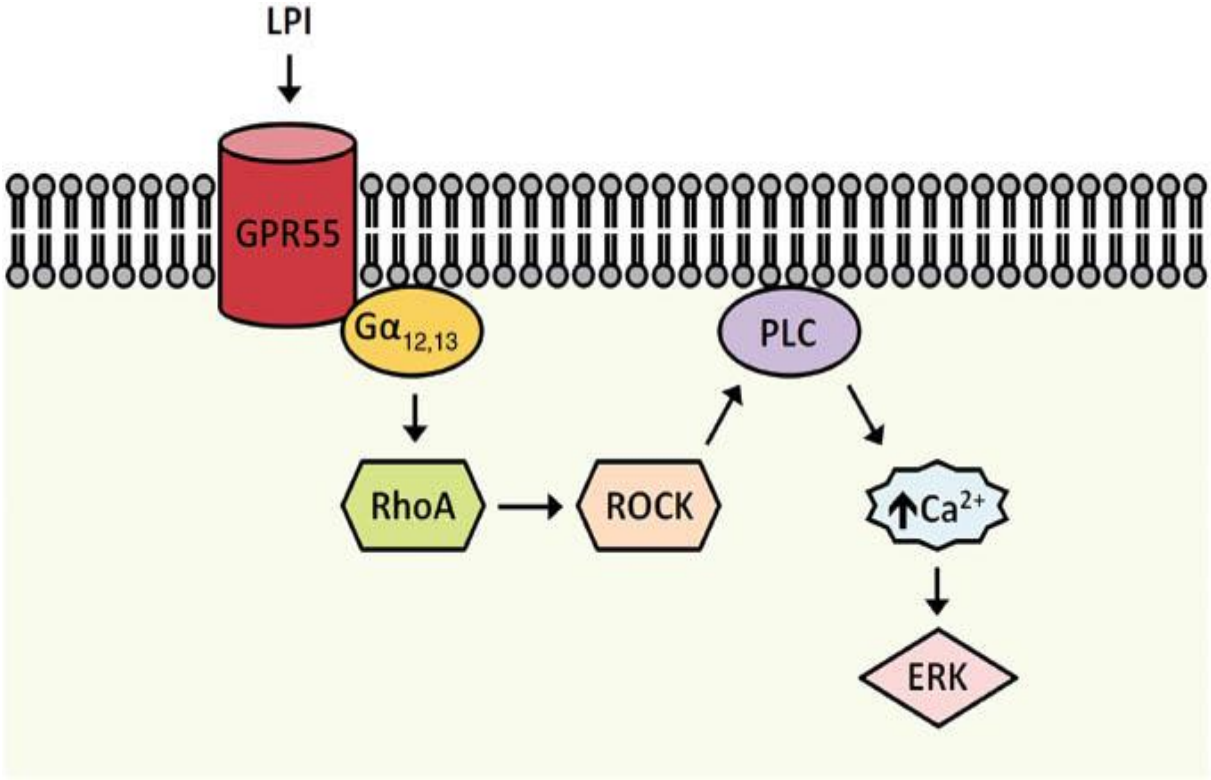


Figure 6: Overview of the GPR55 signaling pathway by LPI. Extracellular LPI can bind and activate GPR55. Activation of GPR55 is coupled to Gα12,13 proteins, thereby activating ras homologue gene family member A (RhoA) and ROCK. ROCK then turns on PLC pathway, which allows cytosolic concentration of Ca^{2+} to increase. Increased intracellular Ca^{2+} in turn leads to ERK phosphorylation and gene expression. (Yang et al., 2015)

The transient receptor potential vanilloid 1 (TRVP1), a ligand-gated cation channel and a member of the transient receptor potential channel family receptor, is also a candidate cannabinoid receptor. TRVP1 receptors are activated by naturally occurring compounds such as capsaicin, vanilloids and anandamine. Its implied role as a cannabinoid receptor is based on the ability of the endogenous cannabinoid anandamide, shown to be structurally similar to capsaicin, to bind and activate this receptor. (Cabral et al., 2009)

3.1.3 Function

To understand the molecular mechanism of cannabinoid signaling in neurons it is important to establish the CB1 functions either as a presynaptic receptor or postsynaptic receptor or both.

In 1999 Katona et al. observed the CB1 immunoreactivity and showed that the reactivity, detected in the hippocampal interneurons, was found to be in the Golgi apparatus and not in the cell surface membrane, indicating that the CB1 corresponds to newly synthesized receptors prior to translocation out of the somato-dendritic compartment into the axonal compartment, implementing that the CB1 receptor is possibly exclusively a presynaptic receptor. (Elphick et al., 2001)

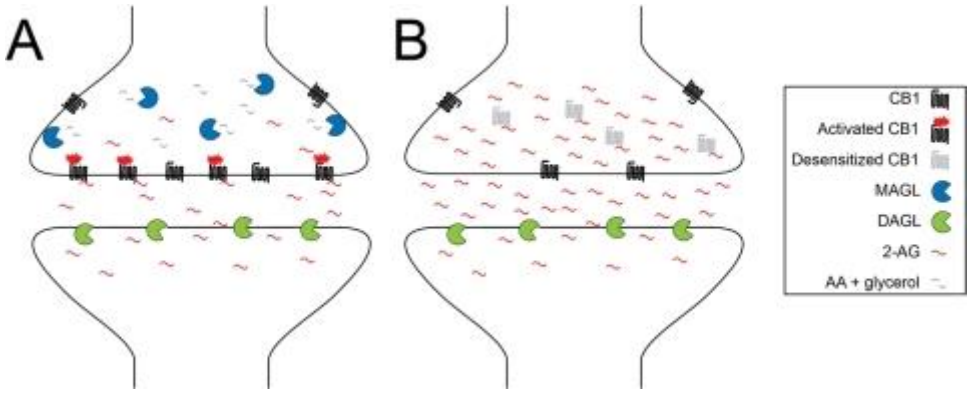


Figure 7: A model for 2-AG signaling at CB1 receptors in the nervous system.

A, under normal conditions, 2-AG is synthesized postsynaptically by diacylglycerol lipase (DAGL), traverses across the synapse to activate CB1 receptors located presynaptically, and then is rapidly inactivated by MAGL.

B, after long-term MAGL blockade, elevated 2-AG levels cause tonic activation and eventual internalization and down-regulation of CB1 receptors.

(Lichtman et al., 2010)

3.1.4 Pharmaceutical Usage

a. Anti-inflammatory effect

In the late 1990s (Zhu et al., 1998) THC was shown to induce apoptosis in macrophages. Furthermore, by inhibiting the production of proinflammatory IL-12, IL12 receptor and interferon-γ, THC suppresses TH1 and increases the expression of anti-inflammatory IL-4, a TH2 cytokine, via CB1 and CB2 receptors. (Newton et al., 1994)

An alternation towards TH1 has been associated with disease progression, whilst a T-cell response towards a TH2 has been linked with therapeutic benefit in MS and EAE. (Hemmer et al., 2002)

There are a few studies showing that, by inducing apoptosis or modulating proliferation in lymphocytes, cannabinoids may suppress the immune and the inflammatory response. Berdyshev et al. demonstrated in 1997 that anandamide inhibits proinflammatory TNF-α production, inhibits IL-6 and IL-8 in LPS-stimulated monocytes and inhibits cytokine soluble receptors. 3 years later Malfait et al. proved that Cannabidiol, the non-psychoactive cannabis compound, causes a dose-dependent suppression of lymphocyte proliferation.

CB2 receptor activation inhibits the functions of immune cells via the known CB2 receptor intracellular signaling mechanisms: inhibition of adenylyl cyclase activity by Gi/o proteins, decreased activity of protein kinase A and NF-κB transcription factor**.** (Walter et al., 2004)

Surprisingly the effects of plant, endogenous and synthetic cannabinoid compounds on peripheral immune cells can induce the opposite response when it comes to the production of NO from immune cells. Synthetic and plant cannabinoids inhibit NO production (Coffey et al., 1996) whilst the endogenous anandamine increases NO production in human monocytes (Stefano et al., 1996) and macrophages (Stefano et al., 1998).

Since most anti-inflammatory mechanisms are connected to CB2 activation, Lowin and Straub reviewd in 2015 how CB1 agonism or antagonism has similar effects on arthritic inflammation.

First, anti-inflammatory effects of CB1 inhibition were shown in THP-1 macrophages, where rimonabant decreased TNF and increased IL-10 production (Miranville et al.,

2010) and in 2013 Duncan et al. demonstrated that the production of pro-inflamatory cytokine and TLR4 expression in macrophages from CB1 knock-out mice, were reduced.

CB1 agonism on the other hand also implies anti-inflammatory effects on immune cells like decreased activation of T lymphocytes by downregulating IL-2. But direct effects are most prominent when injected into the brain, where CB1 activation decreases the activity of microglial cells via reduction of pro-inflammatory cytokines in mice. (Lowin et al., 2015)

Second, FAAH inhibition can counteract the neuroinflammatory component of RA by activating neuronal CB1. Thus, according to Lowin and Straub the best treatment option for RA might be a combination of a peripherally restricted CB1 antagonist and a FAAH inhibitor raising systemic levels of N-acylethanolamines.

Also an inhibition of the fatty acid binding proteins (FABPs), especially FABP 5 and 7,which serve as intracellular carriers that deliver endocannabinoids and N-acetyl-ethanol amines to their catabolic enzymes such as FAAH, reduces endocannabinoid transport and catabolism in cells and FABP inhibitors produce antinociceptive and anti-inflammatory effects in mice. (Kaczocha et al., 2015)

b. Adipositas

It is now well accepted that CB1 activation by Δ^9-THC or synthetic CB1 agonists stimulates appetite.

Since CB1 receptors are expressed in many peripheral organs involved in energy metabolism, including striate muscle and adipose tissue Arrabal et al. demonstrated in 2015 that the expression of relevant metabolic enzymes involved in the regulation of glucose (GPI, TPI), pyruvate (Enolase, PKM1, LDHa), glyoxalase-1 (Glo1) and amino acid catabolism (DLD) pathways in the abdominal rat muscle was modified and showed a complete normalization after the systemic administration of a CB1 receptor antagonist in obese rats.

However, CB1 receptors are also located in the mouse neuronal mitochondria and are able to directly modulate neuronal energy metabolism. Hence Arrabal et al. suggested that CB1 receptor might directly regulate mitochondrial muscle metabolism by targeting the pyruvate dehydrogenase activity, probably partaken by DLD.

Furthermore, CB1 is also present in mature white adipocytes. (Roche et al., 2006)

As shown in Figure 8 the in vitro effect of CB1 activation in these cells includes the increase of glucose uptake, lipoprotein lipase (LPL) and fatty acid synthase (FAS); the stimulation of PPARg expression and adipogenesis and the inhibition of cAMP release, adenosine monophosphate-activated protein kinase (AMPK) and inhibition of adiponectin. (Silvestri et al., 2013)

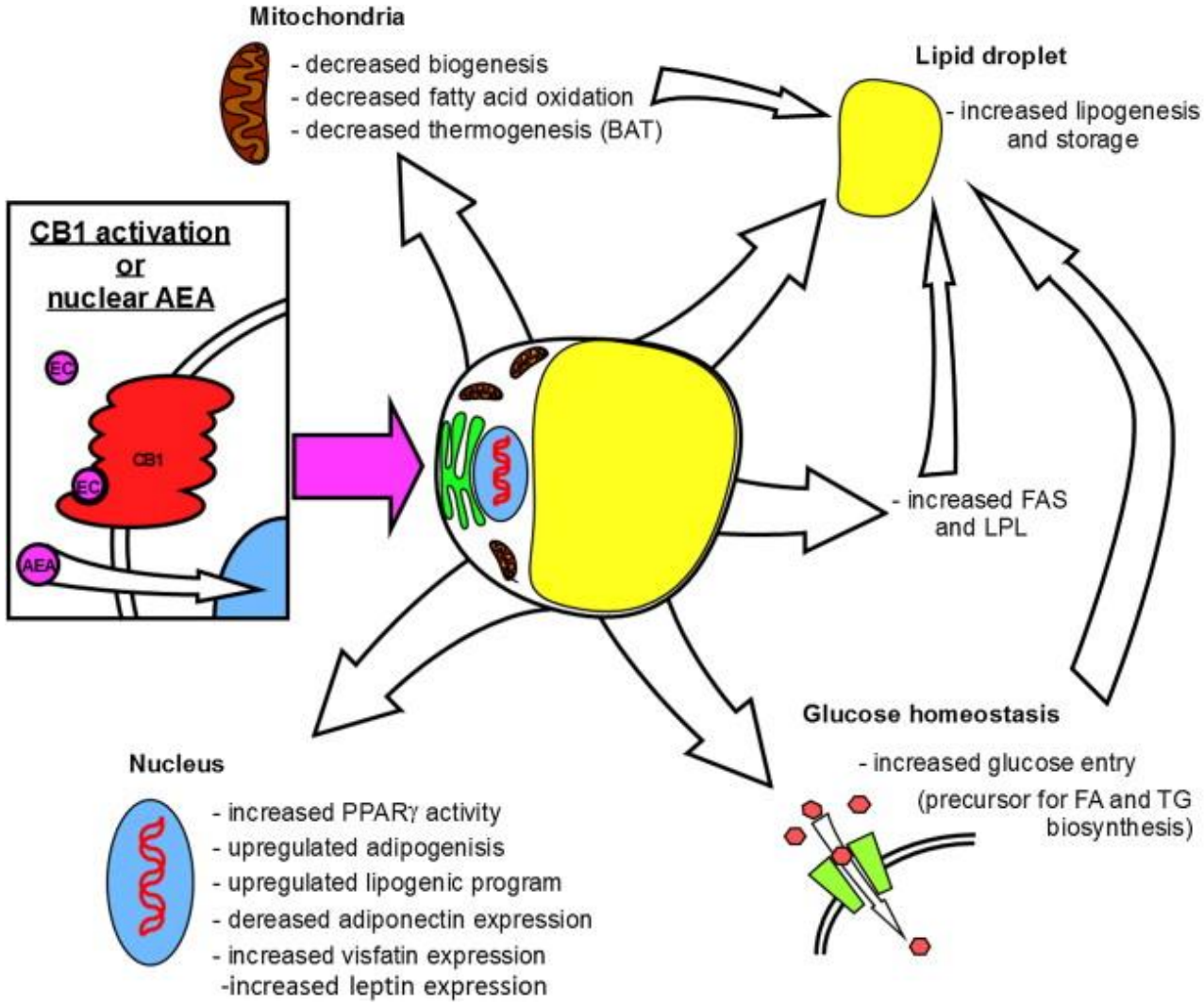


Figure 8: Endocannabinoid Function in the Adipose Tissue

AEA, anandamide; BAT, brown adipose tissue; EC, endocannabinoid; FA, fatty acid; FAS, fatty acid synthase; LPL, lipoprotein lipase; PPARg, peroxisome proliferator-activated receptor-g; TG, triglyceride. (Silvestri et al., 2013)

A popular antiobesity drug, rimonabant, is a CB1 antagonist and inhibits the orexigenic effect of ghrelin (Kola et al., 2008) but because of the consequences associated with its central side effects, the pursuit to develop similar drugs was abandoned.

As mentioned before the CB2 receptor is well known for its immune modulating role but in fact it is also located in the metabolically active tissue. Verty, Stefanidis, McAinch, Hryciw and Oldfield investigated in 2015 the effect of CB2 receptor stimulation on body weight and food intake using DIO (diet-induced obese) mice.

The results showed that the treatment reduced plasma insulin levels and triglyceride levels which are obesity associated metabolic parameters. Furthermore, the data demonstrated the reduction of AST blood concentration, a metabolic enzyme expressed in the liver which is associated with non-alcoholic fatty liver disease (NAFLD) that occurs with increasing obesity, leading to the possibility that CB2 receptor activation could perform a role in protecting the liver from damage in obesity.

c. Alzheimer/Parkinson

Several findings indicate that the activation of both CB1 and CB2 receptors by natural or synthetic agonists, at non-psychoactive doses, have beneficial effects in Alzheimer and confer neuroprotection against Aβ.

The review done by Aso et al. in 2014 summarizes the main studies which demonstrate the effect of cannabinoid compounds for the treatment of AD, thus encouraging the progress towards a clinical trial.

Figure 9 shows that cannabinoids target several processes that play key roles in AD, like Aβ and tau aberrant processing, mitochondrial dysfunction, excitotoxicity, oxidative stress, neuroinflammation, hence the ECS has been demonstrated to modulate the main pathological processes occurring during the period of the neurodegenerative process, including protein misfolding.

So far a few studies described the alteration for CB1 receptors in AD but the results are ambiguous whilst there is no argument regarding the significant increase of CB2 levels in AD brains, primarily connected to receptors expressed on microglia surrounding senile plaques (Ramírez et al., 2005) which supports Aβ transport through the choroid plexus (Martín-Moreno et al., 2012) and clearance of Aβ by macrophages. (Wu et al., 2013)

Bachmeier et al. showed in 2013 that CB receptor agonism or the inhibition of endocannabinoid-degrading enzymes significantly enhanced Aβ clearance across the blood brain barrier.

The neuroprotective benefits of cannabidiol were shown by Iuvone et al. in 2004 reporting that CBD reduced, concentration-dependent, Aβ-induced ROS accumulation and lipid peroxidation, thus pointing out the role of CB2 receptors in reducing oxidative stress damage.

Furthermore, cannabidiol impairs the transcription of NF-κB signaling, hence reduces nitrite (NO) concentration, a oxidant reactive molecule, as well as the expression of iNOS, one of the enzymes responsible for the synthesis of NO. (Esposito et al., 2011)

Nowadays, the accepted treatment for AD is based on acetylcholine esterase (AChE) inhibitors, which increases the Ach concentration or non-competitive antagonists of the N-methyl D-aspartate (NMDA) receptor, which reduces the calcium influx.

Interestingly, Δ^9-THC competitively inhibits AChE (Eubanks et al., 2008) and the synthetic cannabinoid HU-211 acts as an inhibitor of NMDA receptors. (Nadler et al., 1993)

Finally, a couple of studies have demonstrated that certain cannabinoids produce vasodilatation of brain blood vessels and increase cerebral blood flow (Iring et al., 2013) leading to the conclusion that the effect of cannabinoids on the regulation of cerebral blood flow may contribute to their potential benefits on AD.

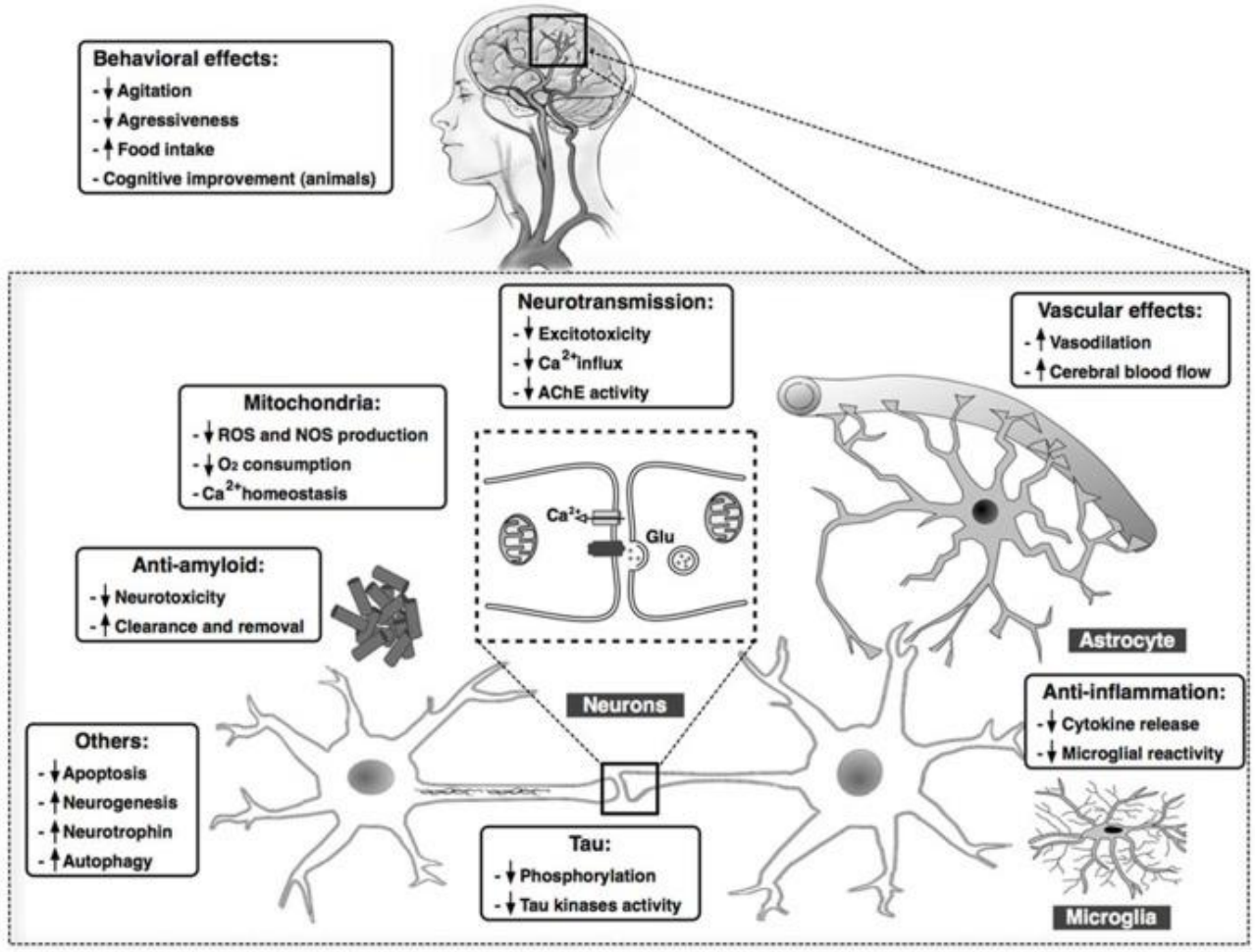


Figure 9: Summary of the main findings demonstrating beneficial effects of cannabinoid compounds in AD models. (Aso et al., 2014)

Based on the evidence that the components of the endocannabinoid system, CB1 CB2 and TRPV1-receptor, are highly expressed in the basal ganglia wherein they interact with dopaminergic, glutamatergic, and GABAergic signaling systems reassess the existing evidence suggesting the involvement of the endocannabinoid system in the treatment of Parkinson. In 2015 More & Choi summarized the pharmacological effects of cannabinoids in various model of PD:

(i) Cannabis was found to improve tremor, rigidity and bradykinesia in PD patients. Also, sleep and pain scores were improved, (ii) WIN-55,212-2 ameliorated L- DOPA induced AIMs (abnormal involuntary movements) and (iii) increased survival of DA neurons in the substantia nigra, reduced expression of pro-inflammatory cytokines and improved motor function, just to mention a few effects.

3.2 Solubility Problem

Solubility, the act of dissolution of a solute in a solvent to give a homogenous system, is an important parameter influencing drug permeation across biological membranes to achieve a certain concentration of a drug in systemic circulation for desired pharmacological response. (Savjani et al., 2012)

Drugs must be able to be pharmacologically active and to permeate biological membranes via passive diffusion, thus they have to possess some degree of aqueous solubility and need to be lipophilic as well.

The reduced bioavailability of oral administered THC, due to its high first pass effect and extremely low water solubility which is limited by its high lipophilicity, necessitating the use of a solubilizing agent such as ethanol or chloroform. (Pertwee et al., 2000)

This difficulty is also hindering the full exploitation of cannabinoids as therapeutic agents, particularly when the delivery of the drug has to be by injection, by aerosol inhalation or topical for ophthalmic use. The therapeutic agents must possess optimum hydrophilic and hydrophobic characters. (Hippalgaonkar et al., 2011)

Therefore there is a need for water-soluble cannabinoid receptor ligands.

3.3 Solubility Agents : Cyclodextrins

Cyclodextrins have mainly been used in the pharmaceutical industry as complexing agents for poorly water-soluble drugs to increase their aqueous solubility, and thus increase their bioavailability and chemical stability.

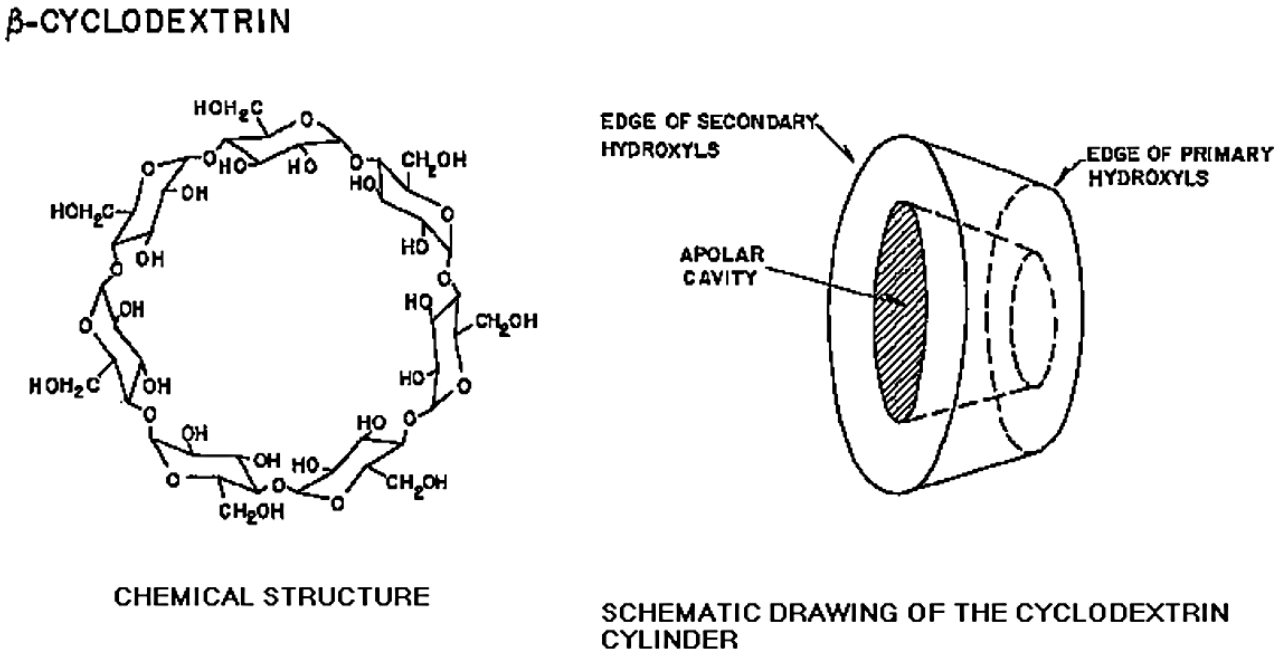


Figure 10: The chemical structure and the schematic drawing of the cylinder of the β-cyclodextrin molecule. (Loftsson et al.; 2005)

They are shaped like cones made of oligosaccharides with a hydrophilic outer surface consisting of (α-1,4-)-linked α-D-glucopyranose units and a lipophilic central cavity.

They can form water-soluble complexes with lipophilic drugs, which ‘hide’ in the cavity. During the complex formation no covalent bonds are formed or broken and the free molecules in the solution are in fast equilibrium with the drug molecules in the complex.

The driving forces for the complex formation include release of enthalpy-rich water molecules from the cavity, hydrogen bonding electrostatic interactions, hydrophobic interactions, van der Waals interactions, and charge-transfer interactions. (Loftsson et al.; 2005)

The molecules are large with a MW ranging from 1000 to over 2000 Daltons with a numerous of hydrogen donors and acceptors and, thus, in general they do not permeate lipophilic membranes. (Khuntawee et al. 2015)

The most common natural cyclodextrins consist of six (α-cyclodextrin), seven (β-cyclodextrin) and eight (γ-cyclodextrin) glucopyranose units (figure 11). The aqueous solubility of β-cyclodextrin is rather limited due to relatively strong binding of the cyclodextrin molecules in the crystal state. (Loftsson et al., 1996)

In general, the CDs must be able to bind the drug strongly enough to deliver it efficiently to the desired target, while at the same time the affinity to guest molecules must be weak enough to allow the drug to be released upon its arrival at the site.

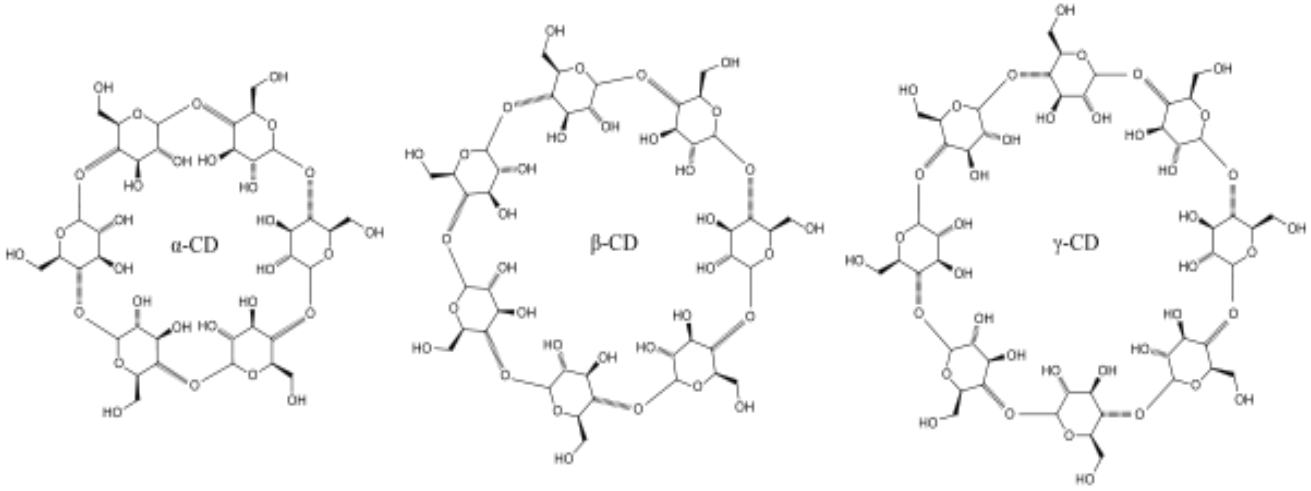
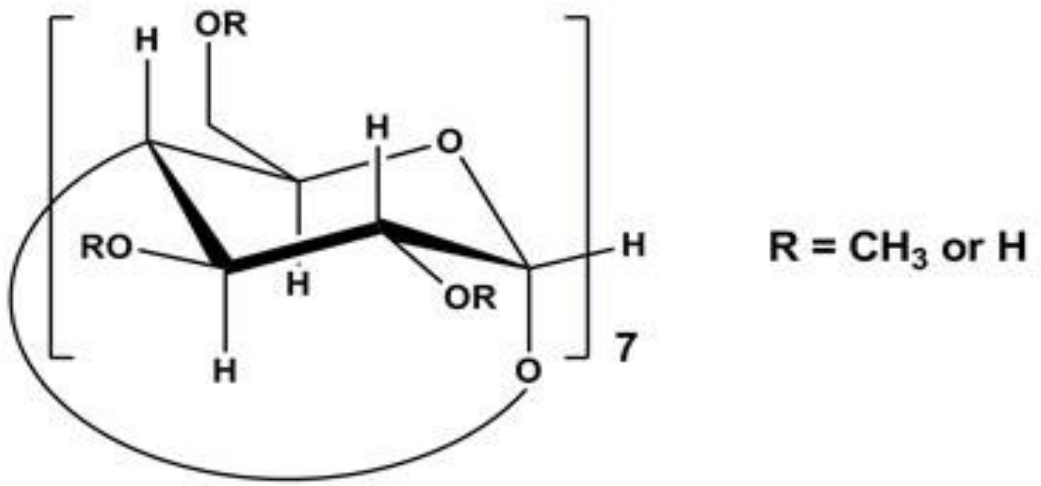


Figure 11: Structures of α-, β- and γ-CD. (Wang et al., 2016)

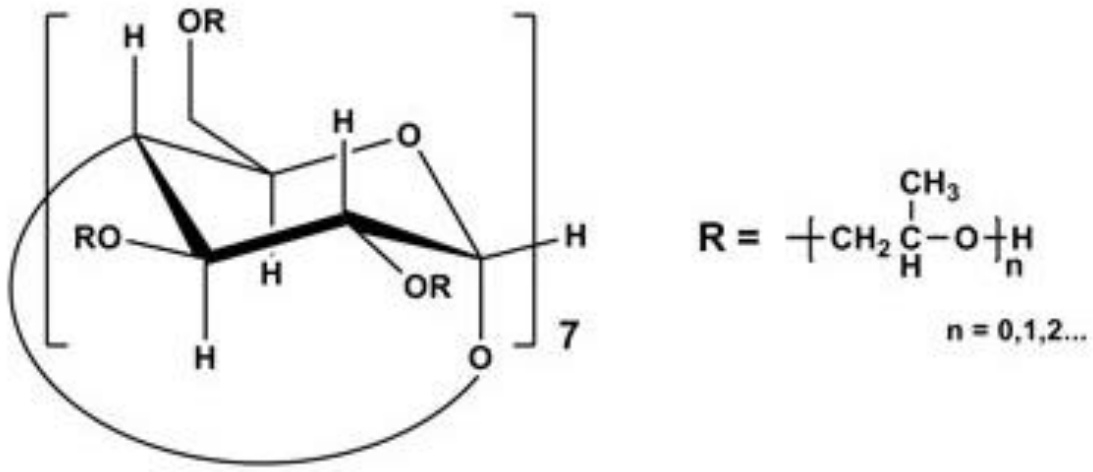
Worldwide over 30 different pharmaceutical products containing cyclodextrins are on the market in different types of formulations, including oral capsules, parenteral solutions, suppositories, nasal sprays, eye drop solutions and dermal products such as Diclofenac sodium, an eye drop solution in complexation with 2-hydroxypropyl-γ-cyclodextrin or Omeprazol (oral administration with β-cyclodextrin). (Loftsson et al., 2002)

Only α-cyclodextrin and the soluble derivatives of β- and γ-cyclodextrin can be used in parenteral formulations because the natural γ-cyclodextrin forms visible aggregates in aqueous solutions and the natural β-cyclodextrin is nephrotoxic. (Loftsson et al., 2005)

Various modifications of the natural CDs have been developed, such as the randomly methylated- β -CD (RAMEB)



and hydroxypropyl(HP)- β-CD. (www.wacker.com)



Mannila et al. (2005) showed that the complexation with RAMEB increases both the dissolution rate and aqueous solubility of THC and based on phase-solubility data a binding ratio of 1:2 (guest:CD) was suggested for the complex. (Hazekamp et al., 2006)

In this study the aqueous solubility of nabilone was tested following binding to HP-β-CD and RAMEB. The resulting complex was complemented by UV studies in order to obtain details on the stoichiometry of the complexation. Concentration stability of the complex was tested up to 4 weeks.

3.4 Nabilone

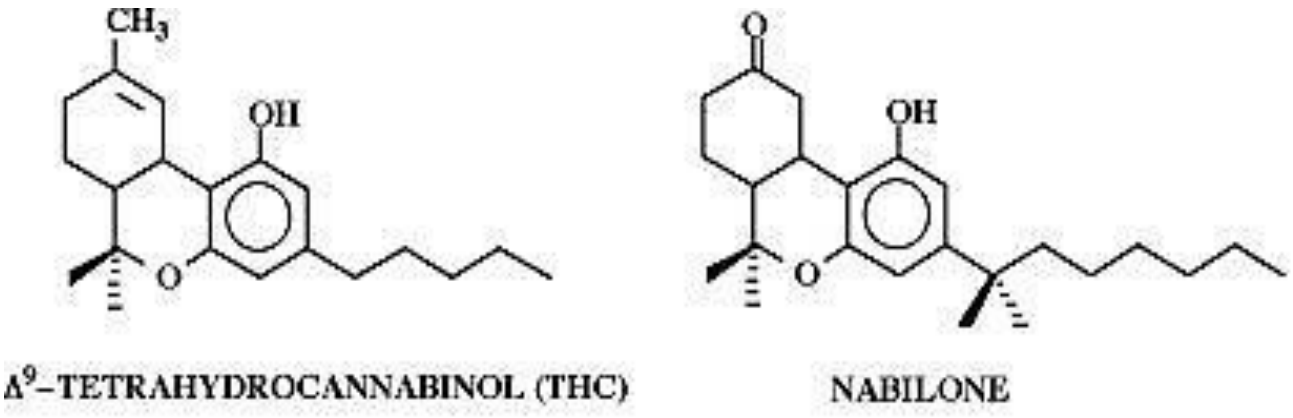


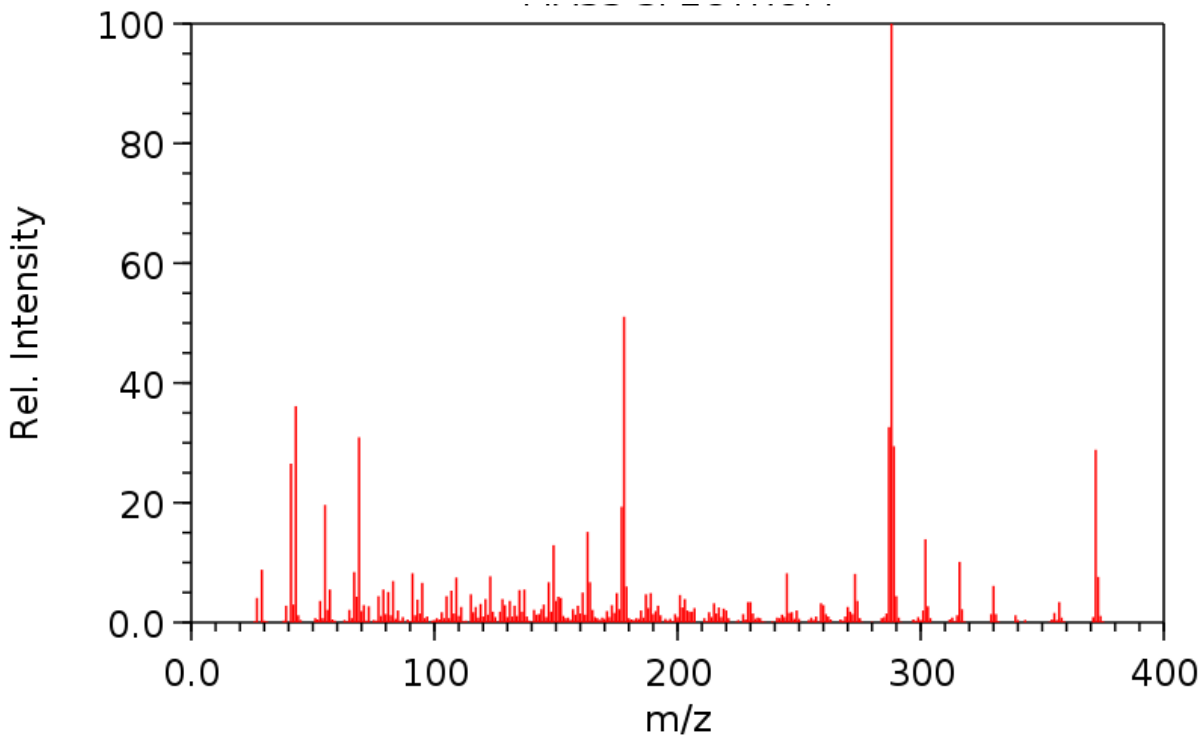
Figure 12: THC and the synthetic analogue nabilone. (Burstein et al., 2009)

Chemical and Physical Properties

Nabilone is a racemate consisting of the (S,S) and the (R,R) isomers.

- IUPAC:
  (6aR,10aR)-1-hydroxy-6,6-dimethyl-3-(2-methyloctan-2-yl)-7,8,10,10a-tetrahydro-6aH-benzo[c]chromen-9-one
  (6aS,10aS)-1-hydroxy-6,6-dimethyl-3-(2-methyloctan-2-yl)-7,8,10,10a-tetrahydro-6aH-benzo[c]chromen-9-one
- SMILES Code:
  CCCCCCC(C)(C)C1=CC2=C(C3CC(=O)CCC3C(O2)(C)C)C(=C1)O
- Molecular Weight: 372.54 g/mol
- Molecular Formula: C24H36O3
- CAS Registry Number: 51022-71-0 (PubChem CID 5284592)

Mass Spectrum (electron ionization)



(NIST Mass Spectrometry Data Center. NIST MS number 352968)

NIST Number	1054459
Instrument Type	IT/ion trap
Collision Energy	0
Spectrum Type	MS2
Precursor Type	[M+H]+
Precursor m/z	373.2737
Total Peaks	15
m/z Top Peak	356
m/z 2nd Highest	357
m/z 3rd Highest	354.2

(PubChem CID 5284592)

Pharmacokinetics and pharmacodynamics

The white to off-white polymorphic crystalline substance is an orally active synthetic cannabinoid which, like other cannabinoids, has complex effects on the central nervous system (CNS).

It appears to be completely absorbed from the human gastrointestinal tract when administered orally, it gets metabolized by multiple P450 enzyme isoforms and the major excretory pathway is the biliary system. The biological half-life for nabilone and its metabolites are 2 to 35 hours and exhibits dose linearity within its therapeutic range.

Tolerance to these effects develops rapidly and is readily reversible.

In aqueous media, the solubility of nabilone is less than 0.5 mg/L, with pH values ranging from 1.2 to 7.0. (www.drugs.com)

In 1985 nabilone (Cesamet) was approved by the U.S. Food and Drug Administration (FDA) for the treatment of chemotherapy-induced nausea and vomiting that has not responded to conventional antiemetics. Clinical trials evaluated Cesamet for its effectiveness and safety in the treatment of nausea and vomiting induced by cancer chemotherapy in patients receiving chemotherapy, including low- dose cisplatin (20 mg/m^2) in both placebo-controlled and active controlled (prochlorperazine) trials. (Figure 13a/13b)

In Austria nabilone is marketed as Canemes.

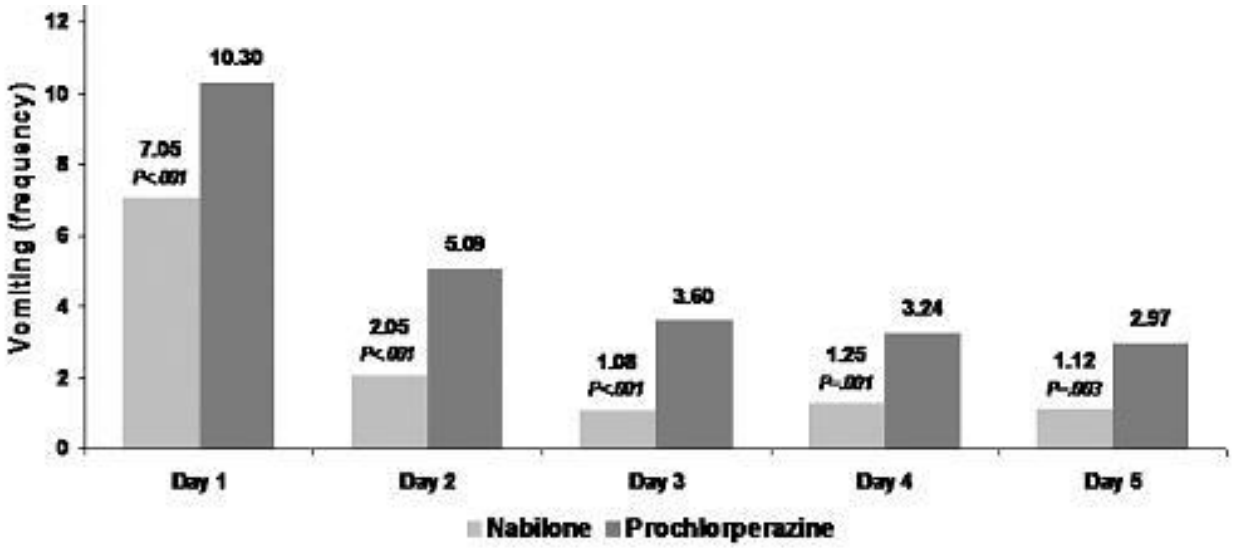


Figure 13a: Nabilone reduces frequency of vomiting on chemotherapy days 1 through 5. (Ware et al., 2008)

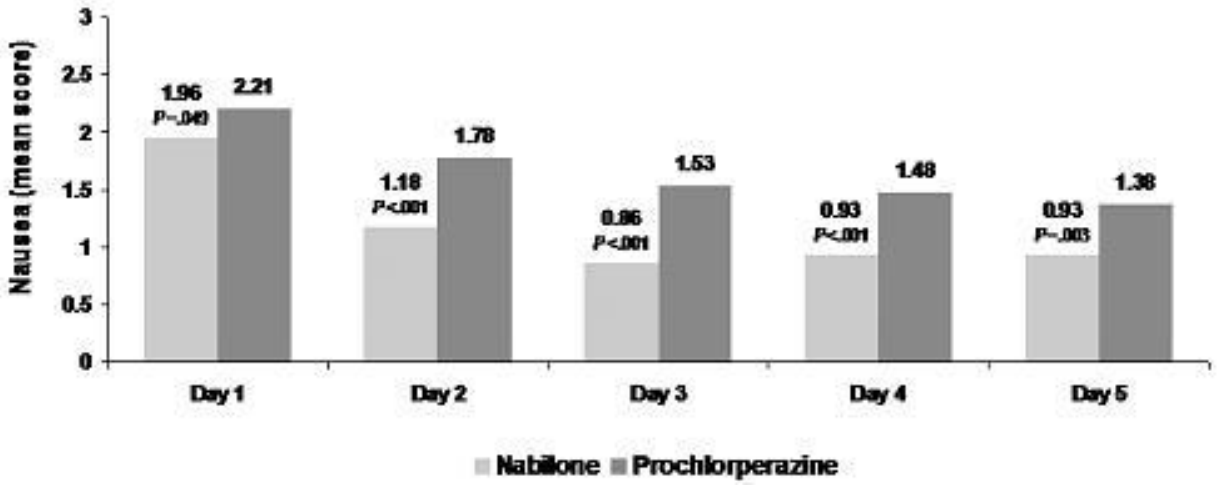


Figure 13b Nabilone significantly reduces the severity of nausea throughout the chemotherapy cycle. (Ware et al., 2008)

As a synthetic cannabinoid its effects on the mental state are similar to those of cannabinoids, thus patients may experience euphoria, detachment, depression, anxiety, panic, paranoia but these side effects appear to be more common when larger doses of Cesamet are administered. (www.drugs.com)

The approved indication, 1-2mg, is administered the night before and one to three hours prior to the chemotherapy and can be continued up to 24 hours following the chemotherapy.

4. MATERIALS AND METHODS

4.1 Materials

Nabilone was provided by LOBA Feinchemie GmbH; Austria.

The Randomly Methylated β-Cyclodextrin (RAMEB) was purchased from Wacker Chemie, Germany, and Hydroxypropyl-β-Cyclodextrin was purchased from Roquette, France.

4.2 Methods

4.2.1 UV-Spectroscopy

UV-spectrometers are used to measure the absorbance of ultra violet or visible light by a sample at a single wavelength or to perform a scan over a range in the spectrum. The UV region is from 190 to 400 nm and the visible region ranges from 400 to 800 nm, within this area we distinguish the colors of the rainbow from violet through to red. The technique can be used both quantitatively and qualitatively.
(Royal society of chemistry. (2009). Ultraviolet/ Visible Spectroscopy)

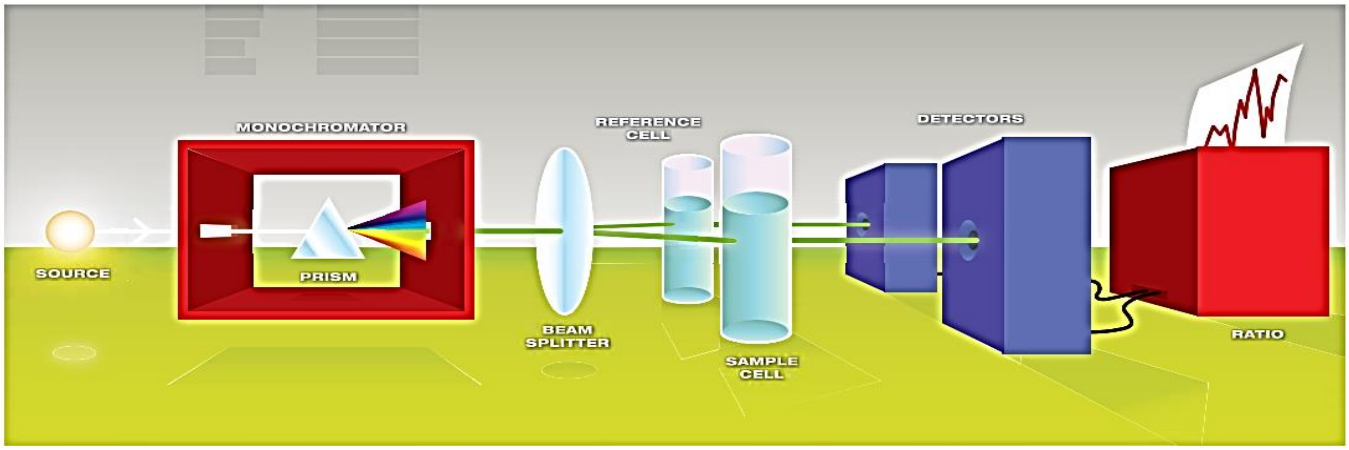


Figure 14: A schematic diagram of a UV-visible spectrometer. (Royal society ofchemistry. (2009). Ultraviolet / Visible Spectroscopy)

It measures the intensity of light after passing through a sample I, and compares it to the intensity of light before it passes through the sample I_0. The ratio I/I_0 is called the transmittance. The absorbance A is based on the transmittance**:** $\boldsymbol{A = \log I_0/I}$

Figure 14 shows the basic parts of a spectrophotometer, which are a light source, a holder for the sample, a prism to separate the different wavelengths of light, and a detector, used to convert the incoming light into a current. The radiation source is a deuterium lamp or a tungsten lamp. Samples are placed in a rectangular cuvette, generally with an internal width of 1 cm. The most widely pertinent cuvettes are made of quartz glass or high quality fused silica because these are transparent throughout the UV, visible and near infrared regions.

It is important that the samples are pure and free from any kind of precipitation.
The Lambert-Beer law claims that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length.
(Royal society of chemistry. (2009). Ultraviolet / Visible Spectroscopy)
Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution.

$$A = \varepsilon * c * l$$

A = absorbance

l = optical path length, i.e. dimension of the cell or cuvette (cm)

c = concentration of solution (mol l^{-3})

ε = molar extinction coefficient, which is constant for a particular substance at a particular wavelength (dm^3 mol^{-1} cm^{-1})

The law is only applicable for monochromatic light, which is light of a single wavelength or narrow band of wavelengths, and provided that the physical or chemical state of the substance does not change with concentration. (Broderick, 2013).

4.2.2 Lyophilization

Lyophilization or freeze drying is a process in which water is frozen, providing a necessary condition for low temperature drying, followed by its removal from the sample, initially by a phenomenon called sublimation, where water passes directly from solid state to the vapor state without passing the liquid state (primary drying) and then by desorption (secondary drying).

Sublimation takes place under a vacuum at pressures and a temperature below triple point, hence it is suited to dry thermolabile compounds. (Nireesha et al., 2013)

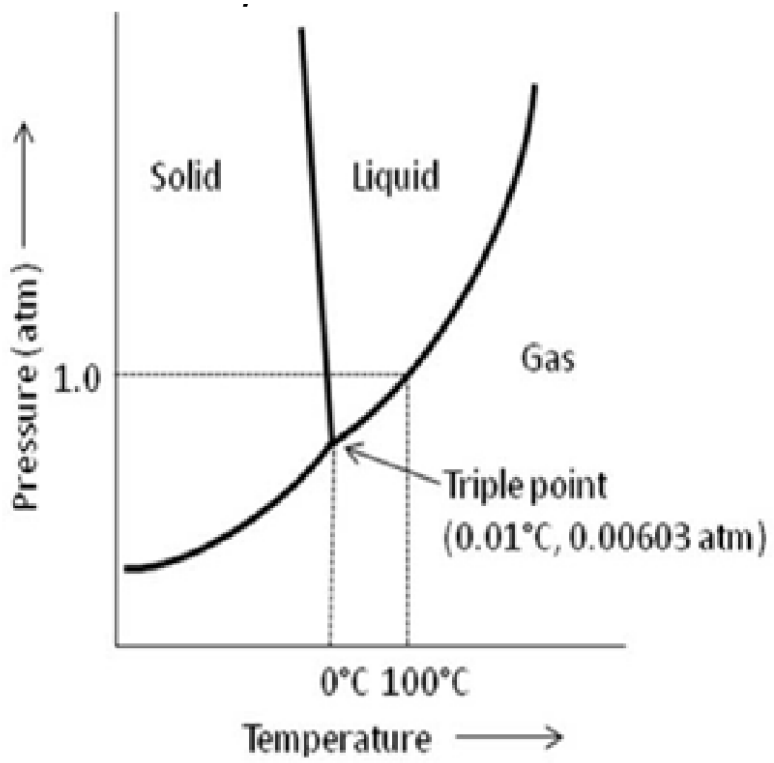


Figure 15: Phase diagram showing the triple point of water at 0.01°C, 0.00603 atm. Lyophilization. (Nireesha et al., 2013)

When almost the whole frozen water is removed via sublimation primary drying is complete but at this point, the product still contains some bound unfrozen water that has to be removed at higher temperatures by desorption, the secondary drying. (Rajeevani et al., 2015)

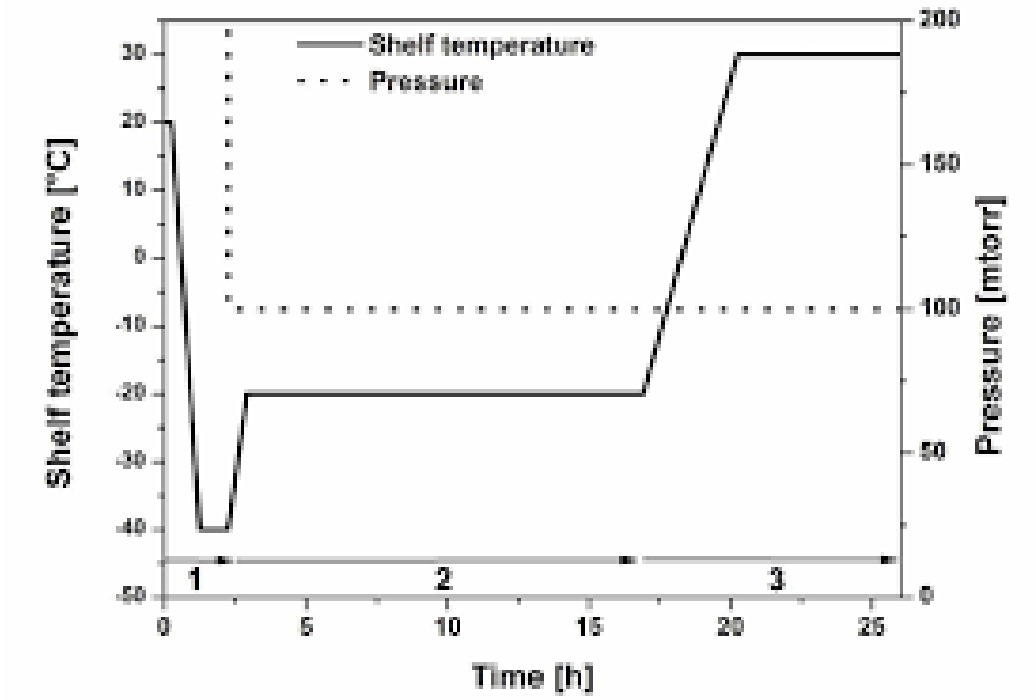


Figure 16: Schematic illustration of a typical lyophilization cycle. 1) Freezing, 2) Primary drying, and 3) Secondary drying. (Rajeevani et al., 2015)

The most typical use of lyophilization is the manufacturing of parenterals and the process also gained importance for the preservation of nucleic acid based pharmaceuticals.

Furthermore, as many new substances suffer from poor solubility, the preparation of amorphous solid dispersions by lyophilization, especially from co-solvents systems, is a promising way to achieve stable systems with an increased bioavailability and a low tendency to recrystallization or particle size increase. (Rajeevani et al., 2015)

4.2.3 Producing ethanolic soltions

To produce a $5*10^{-3}$ molar nabilone stock solution 93,2mg nabilone was stirred at room temperature with 50ml ethanol and as expected the nabilone solubilized completely in ethanol. Further, 7 different samples were made by diluting an aliquote (1ml) of the stock solution 1:100 with increasing amount of ethanol in the water/ethanol mixture, from 10% up to 100% in steps of 10%. Due to immediate flocculation occurring in the samples, with 10%, 20% and 30% content of ethanol, centrifugation was performed first for about 30 minutes and the clear samples were measured via UV-spectrometer.

Further measurements took place 3 weeks later to test the stability of the nabilone concentration.

4.2.4 Producing a nabilone:CD complex

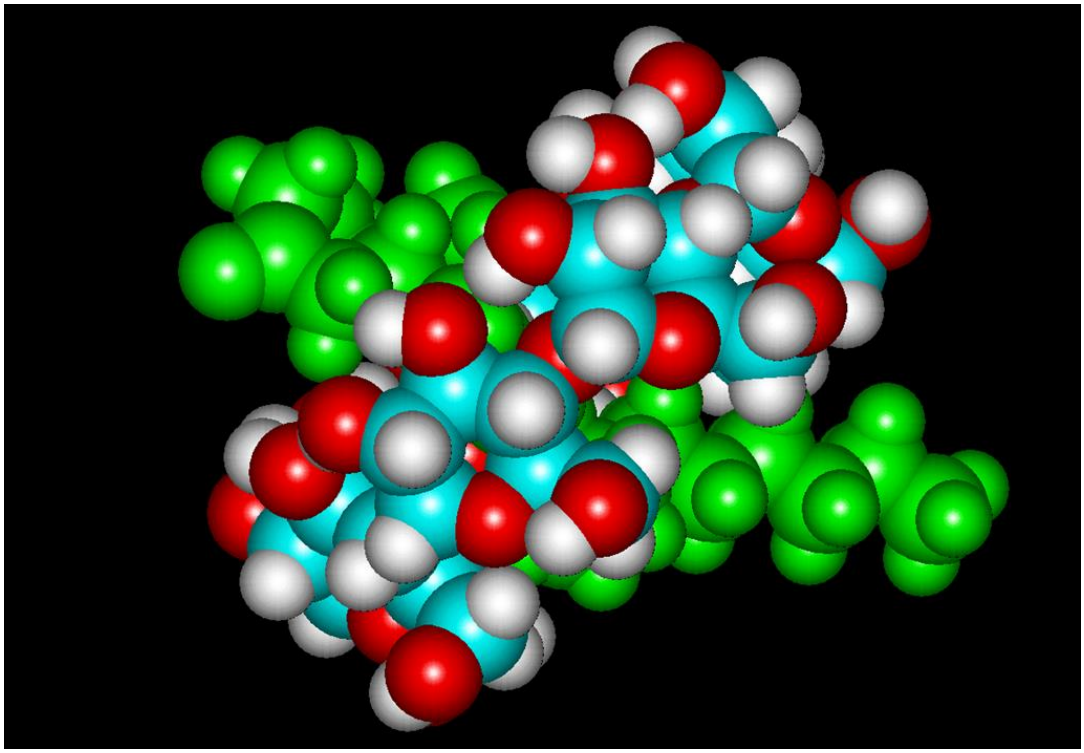


Figure 17: Structure of a 1:1 nabilone (green) cyclodextrin complex.

Two different CDs were used to produce nabilone:CD complexes, HP-β-CD and RAMEB. To perform the time depending behavior test (see point 5.2) an excess amount of nabilone was stirred for 5 hours at room temperature with a 10^{-3} molar concentration of RAMEB (65,6mg/50ml water) and HP-β-CD (70mg/50ml water). Every hour samples from each solution were taken and measured spectrophotometrically.

For the filtration and stability test the nabilone:CD solutions where placed after 2 hours of stirring in a centrifuge for 30 min, then filtrated to avoid altering of the precipitation and afterwards analyzed every 50 hours.

5. RESULTS & CONCLUSION

The results are demonstrated by phase-solubility diagrams since they are useful tools to study inclusion complexes between cyclodextrins and substances with poor aqueous solubility. (Onnainty et al., 2013)

5.1 Ethanolic solutions

The ability of an UV-Spectrometer to detect dissolved nabilone in ethanolic solutions is demonstrated in the figure below.

As already mentioned nabilone is not able to dissolve in sufficient amounts when only water is used as solubilizing agent. No distinctive peak can be seen.

To confirm the influence of increasing percentage of ethanolic solution as a solubilizing agent on the ability to dissolve nabilone scans were performed. For the following measurements a wavelength of 280nm was set.

First the nabilone stock solution was diluted 1:100 with a 10% ethanolic solution but no distinctive peak could be detected.

When a 50% ethanolic solution was used as a solubilizing agent to dissolve nabilone a characteristic peak could be seen. Further measurements followed with a 60% and 80% ethanolic solution leading to the expected results; hence we can come to the conclusion that by increasing the amount of EtOH, the ability to dissolve nabilone rises. (Figure 18)

These diluted solutions where stored safely at room temperature and measured again 3 weeks later. We could notice that the flocculation increased with time especially in the ones where the stocksolution was diluted with a 10%, 20% and 30% ethanolic solutions. Thus, we expected a decreased nabilone concentration and the UV results shown in figure 19 verify this. The UV spectra of particularly these 3 solutions did not show any distinctive peak. Hence, within 3 weeks the concentration of nabilone decreased down to zero. Further, the concentration of nabilone in the 40% ethanolic solution decreased to almost the same amount ($2.02*10^{-6}$ molar) as the 10% solution that was measured 3 weeks earlier.

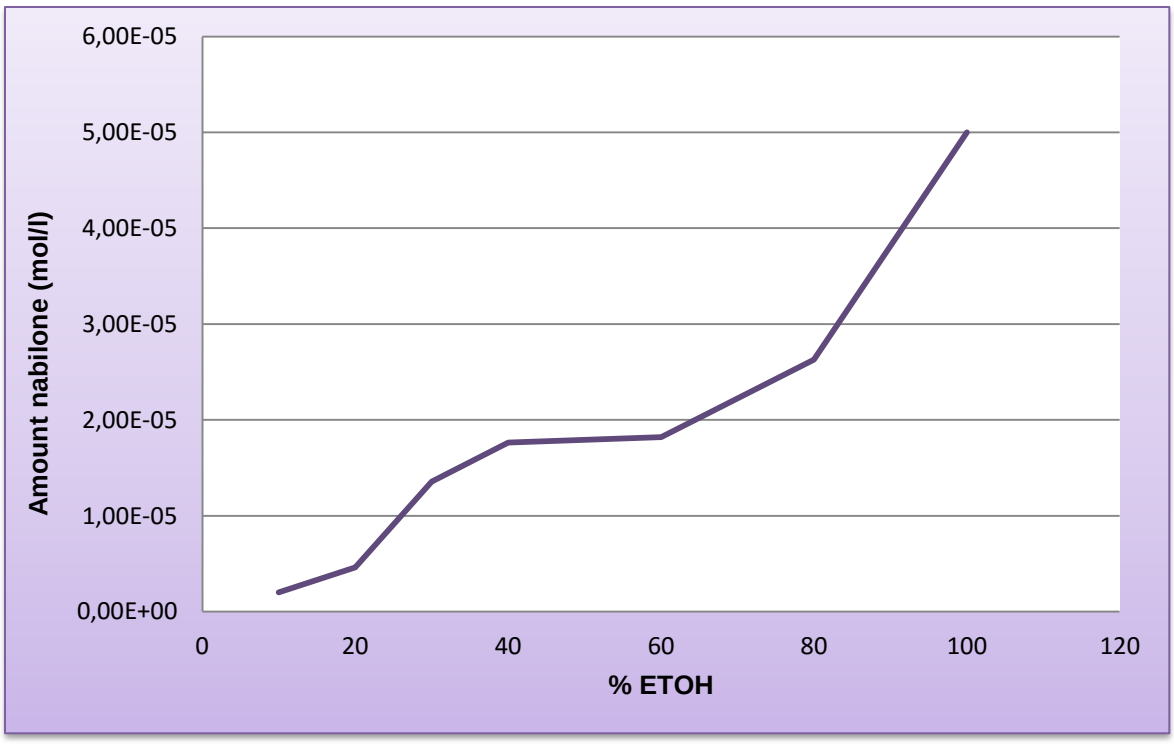


Figure 18: Concentration of nabilone in the presence of water/ethanol mixtures with increasing amount of ethanol in the solutions.

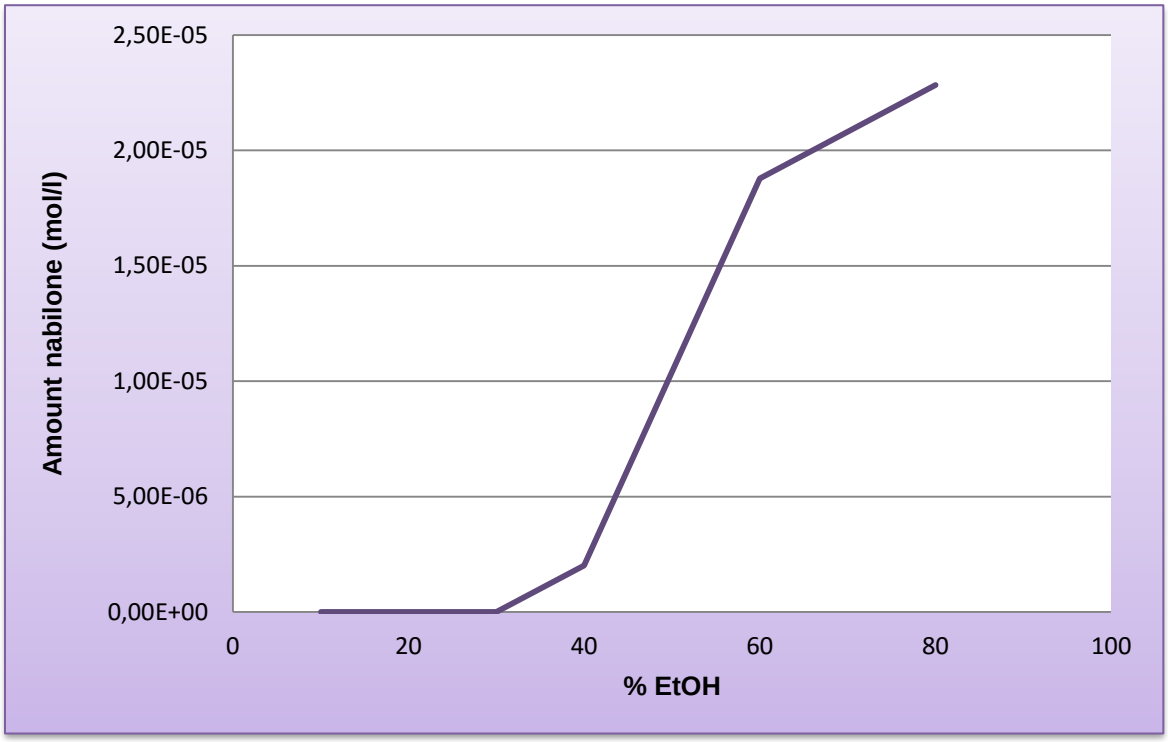


Figure 19: Concentration of nabilone in the ethanolic solutions measured 3 weeks later.

5.2 Time depending behavior

To test the phase-solubility an aqueous solution of a specific amount of the CD was stirred with an excess amount of nabilone for a specific time at room temperature.
To show the behavior of nabilone in a water-CD-solution over a particular period the following experiment was performed.
Nabilone was set on constant agitation for 5 hours each in a 10^{-3} molar concentration of RAMEB (65,6mg/50ml) and HP-β-CD (70mg/50ml) and samples were measured via UV-spectrometer every hour. The results show clearly that CDs improve the aqueous solubility of nabilone, especially in complexation with HP-β-CD. However, as we see in figure 20 the highest concentration of soluble nabilone reaches its peak after 2 hours of stirring and starts reducing afterwards. This test first implied a vague suspicion that, after these particular 2 hours of stirring, the stoichiometric conformation of a 1:1 and 1:2 (nabilone:CD) complex changes in a less soluble complex maybe due to the constant presence of un-dissolved nabilone in the solution. To confirm this thesis, further experiments were performed.

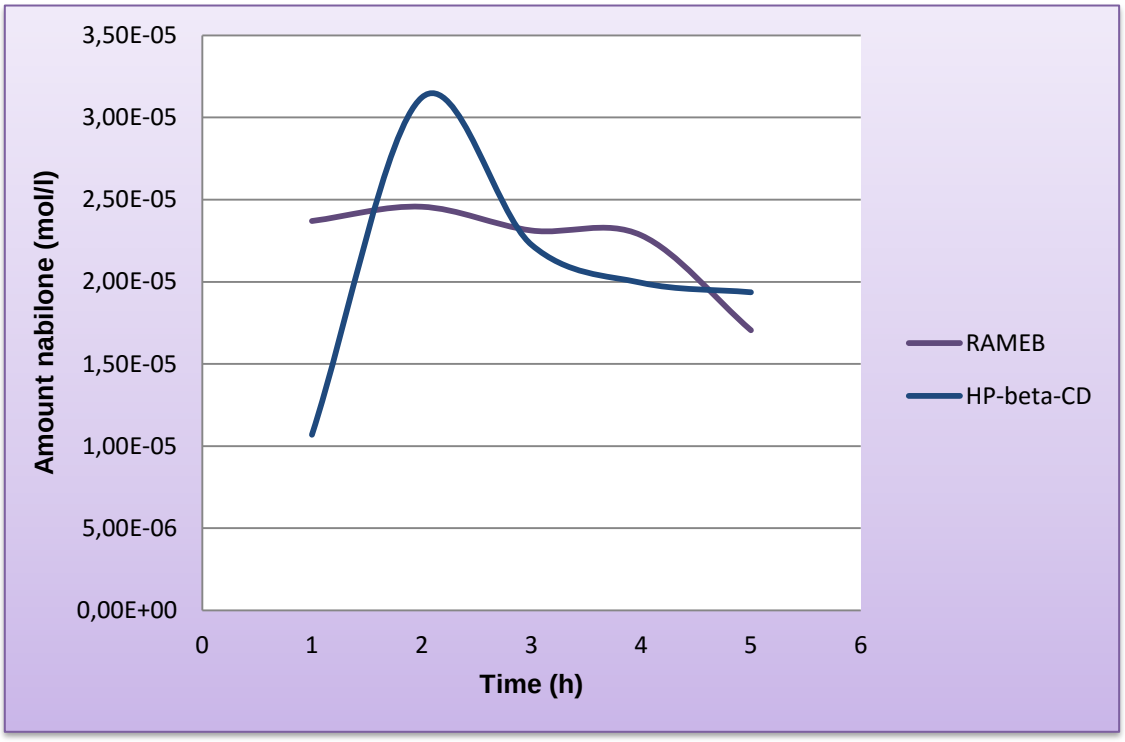


Figure 20: Time depending behavior of nabilone in water-CD-mixtures using a 10^{-3} molar concentration of RAMEB and HP- β-CD for each solution.

5.3 Filtration experiment

As shown in the earlier experiment “time depending behavior” the concentration of soluble nabilone decreases after 2 hours of stirring. The influence of the un-dissolved nabilone and the presence of CD in the solution may lead to a formation of complexes of higher order that are less soluble. The following experiment was an attempt to find out if, after the concentration reaches its peak, a filtration of the solution could have a positive and stabilizing effect on the concentration of the soluble nablione:CD complex.

Again two different solutions were made, one with a 10^{-3} molar concentration of HP-β-CD and in the second one with the same concentration of RAMEB. Nabilone was added to the solutions and stirred for 2 hours. To remove the un-dissolved nabilone the solutions were centrifuged for 30 minutes and then filtrated. Each solution was then measured via UV to detect the concentration of dissolved nabilone.

The two nabilone:CD-complex solutions where stored safely at room temperature and analyzed every 50 hours.

The following figure 21 summarizes and demonstrates the results of this test. We can see that after some oscillations the solutions remain stable. However, this test verifies the theory that by removing un-dissolved nabilone the dissolved complex concentration reaches a stability condition. Thus this confirms the assumption that the presence of un-dissolved nabilone has an effect on the stoichiometric conformation of the complex.

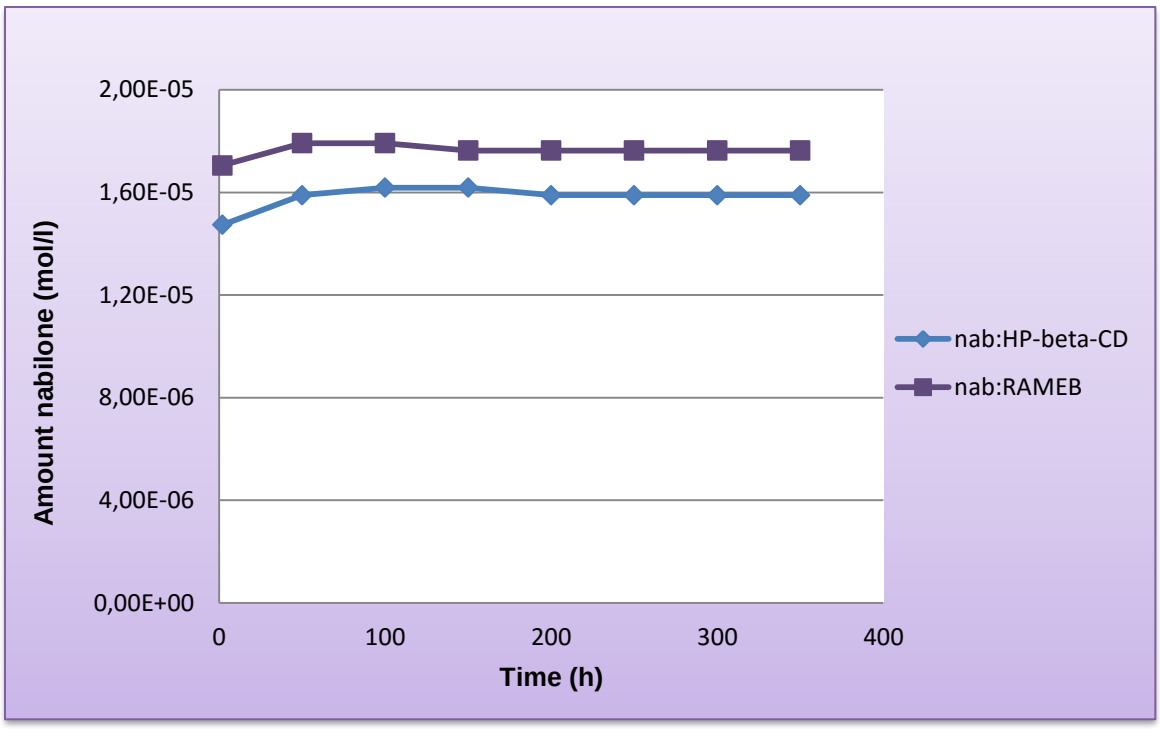


Figure 21: Time depending behavior of nabilone in water-CD-mixtures (using a 10^{-3} molar concentration of RAMEB and HP-β-CD for each solution). After filtrating un-dissolved nabilone from the solutions the concentration remains stable.

5.4 Stability of concentration experiment

Having proved that a stable nabilone:CD-complex concentration could be reached, further investigations on how stable this condition is, were made.

Again, two nabilone:HP-β-CD and nabilone:RAMEB solutions of the same compositions were made, stirred for 2 hours and then centrifuged, filtrated and measured. On the following day, to test the concentration stability of this soluble complex, a small amount of nabilone was additionally added to the centrifuged and filtered clear solutions.

Further measurements took place every 50 hours and the results can be seen in the figures 22a and 22b.

Here the charts show a steady continuous fall of the soluble nabilone:CD complex concentration in the solutions without implying that a condition of stable concentration is going to manifest again.

This significant decrease of concentration could also be an indication for a new stoichiometric less soluble complexation of nabilone with CD.

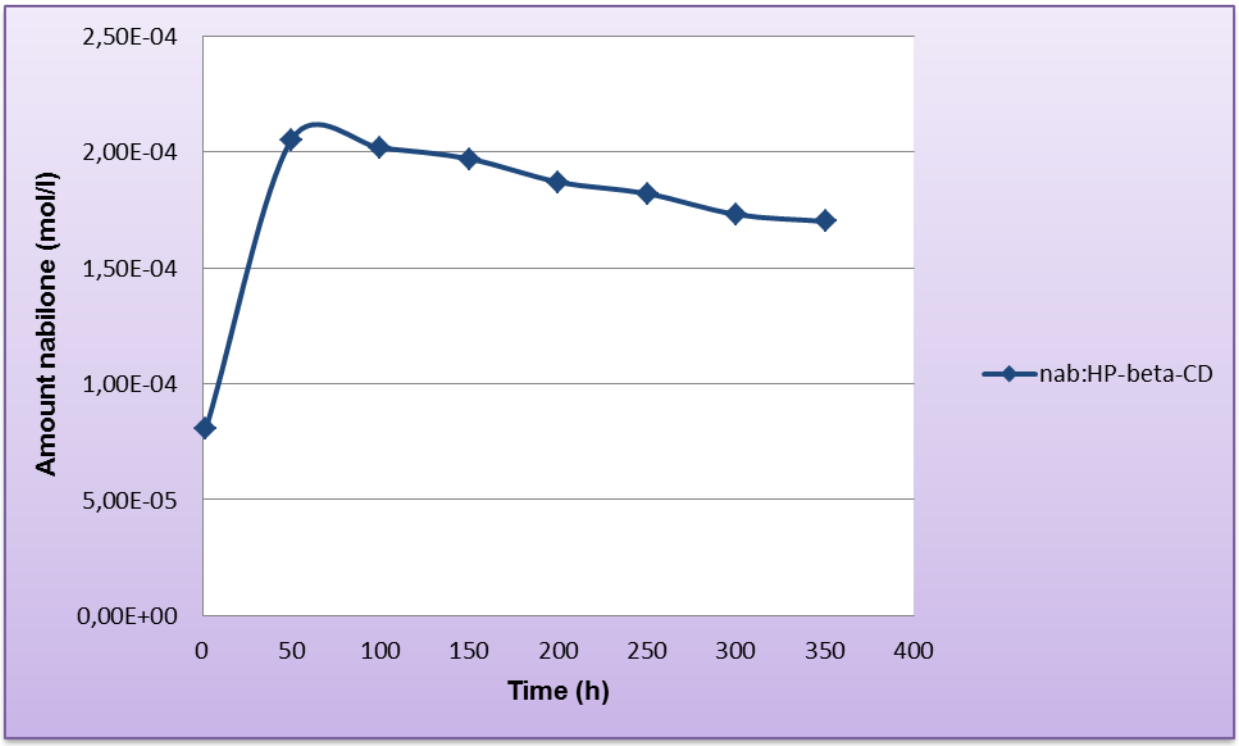


Figure 22a: Time-concentration curve of a nabilone: HP-β-CD solution showing the decrease of concentration due to additionally added excess amount of nabilone.

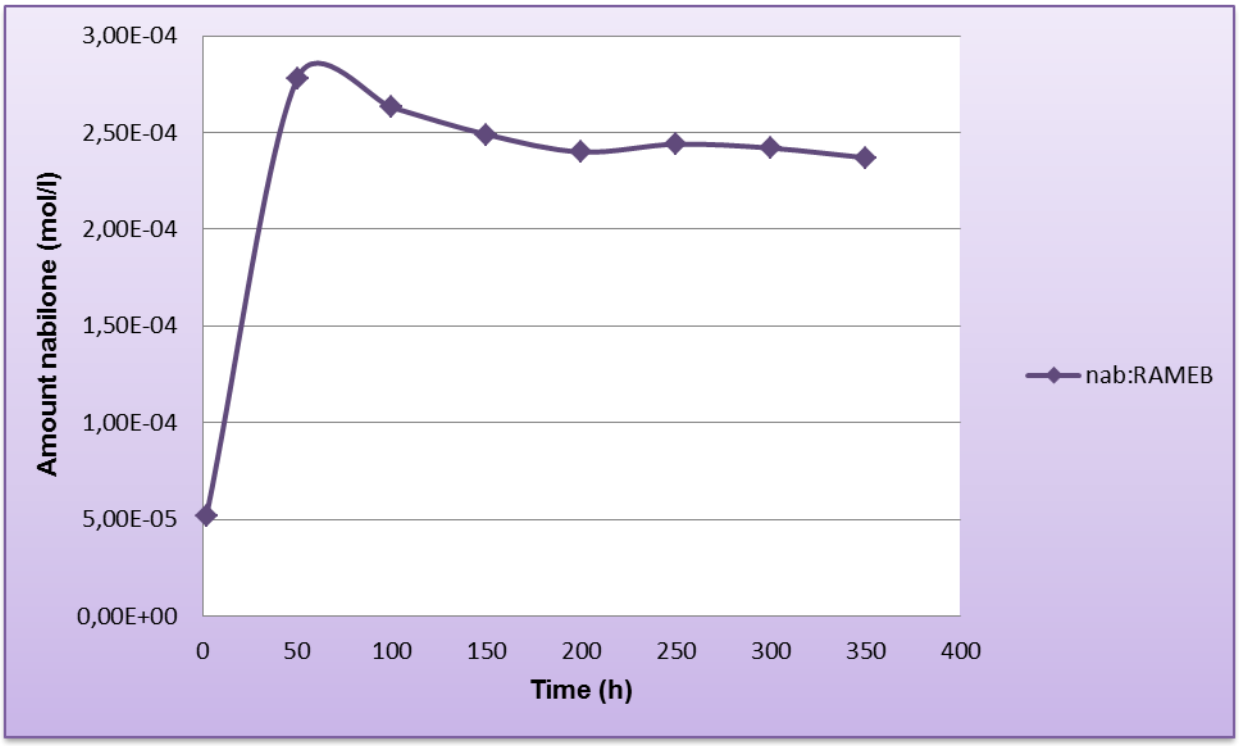


Figure 22b: Time-concentration curve of a nabilone: RAMEB solution showing the decrease of concentration due to additionally added excess amount of nabilone to a.

5.5 Lyophilization test

The lyophilization experiment brought promising results in terms of increasing solubility.

An excess amount of nabilone was mixed with ever increasing concentrations of CD. The tested CD was HP-β-CD.

First, ethanolic stock solutions with nabilone (93,2mg in 50ml ethanol) were made and the samples were evaporated to dryness under vacuum.

Meanwhile, 3 different concentrations of water/CD solutions, each 100ml, were made, a 10^{-4} molar (14mg HP-β-CD), a 10^{-3} molar (140mg HP-β-CD) and a 10^{-2} molar (1400mg HP-β-CD) solution.

The dried samples were resuspended in the prepared water/CD solutions by ultrasonication for 1 hour, then left to equilibrate for 72hours at room temperature under constant agitation.

After equilibration, un-dissolved nabilone was removed from the suspensions by centrifugation and filtration using quartz frits. The clear solutions were frozen overnight, lyophilized and reconstituted in a small quantity of ethanol (3ml) for quantification of dissolved nabilone by UV.

As shown in the figure 23 the amount of CD has a high impact on the solubility of nabilone. The first column (1), representing the10^{-4} molar CD solution, has the smalest amount of soluble nabilone compared to the 2nd (10^{-3} molar) and the 3rd column (10^{-2} molar). This leads to the conclusion that the higher the CD concentration is the more nabilone is able to solubilize.

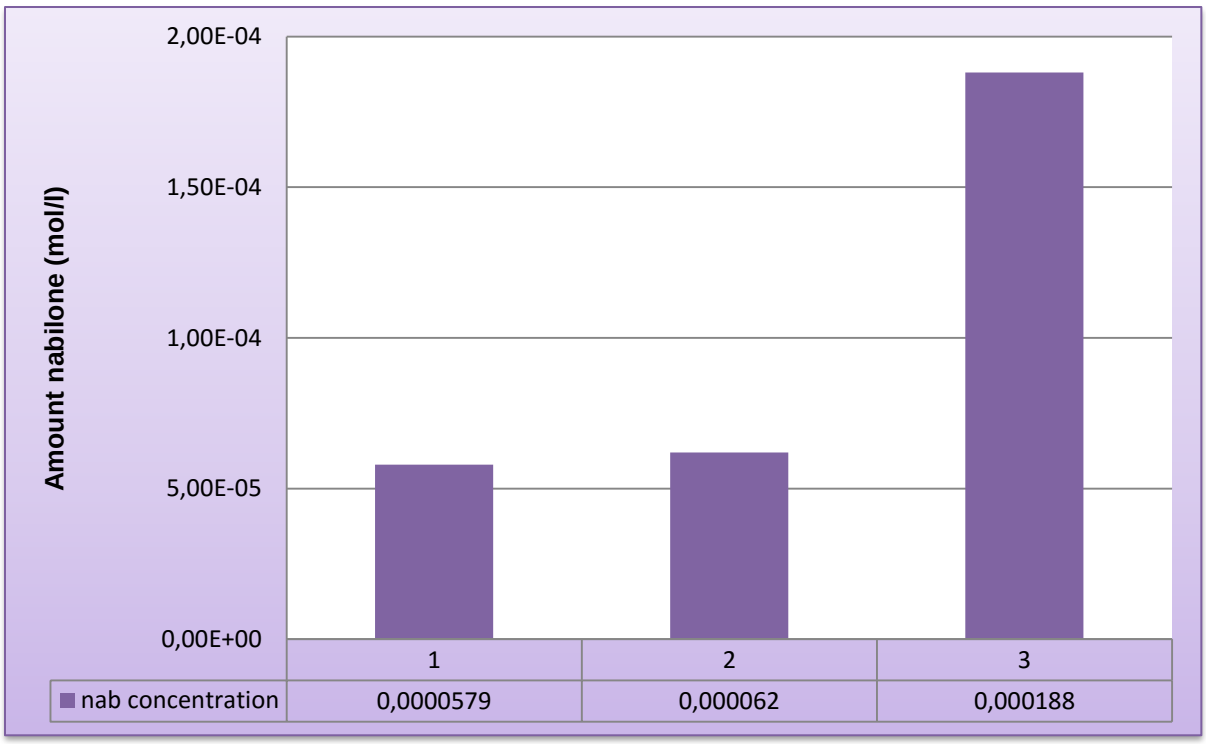


Figure 23: Nabilone concentration set in dependence of HP-β-CD concentration.

1 = 10^{-4} molar CD concentration;

2 = 10^{-3} molar CD concentration;

3 = 10^{-2} molar CD concentration.

The amount of dissolved nabilone rises with the concentration of CD in the solution.

6. ADDITIONAL TEST

6.1 Melting point

A melting point test was performed to compare the precipitants of the solutions used in the stability test, as seen in point 5.4, and to investigate the stoichiometric change in the nabilone:CD complex.

Thus, two fresh nabilone solutions each with a 10^{-3} molar concentration of RAMEB and HP-β-CD were made and stirred for 2 hours. The un-dissolved precipitate was removed from the liquid by filtration and then dried.

These precipitants showed a melting point from 149°C-155°C which indicates un-dissolved nabilone.

As demonstrated previously in 5.4, the presence of un-dissolved nabilone in the solution has an effect on the stoichiometric conformation of the complex. Thus the dried precipitants of the previously made solutions, which contain additionally added nabilone to the filtered clear solutions, seen in point 5.4, were used to compare the melting points.

Here the results were 141°C-145°C, showing that they neither agree with the melting point of nabilone nor with RAMEB or HP-β-CD.

Hence, we have another indication for our theory that the additional amount of nabilone in the former clear solution promotes formation of un-soluble nabilone:CD complexes.

7. SUMMARY

Cannabinoids have shown promising properties as antiemetic and as adjunct analgesic against neuropathic pain. Thus the synthetic cannabinoid nabilone was manufactured for the treatment of chemotherapy-induced nausea and vomiting. However, similar to the natural cannabinoids, the utility of nabilone is limited due to its high hydrophobicity (logP 6,8) and poor aqueous solubility. (Hippalgaonkar et al., 2011)

This major problem encounters with the development of formulation and is a big challenge for pharmaceutical technology.

Nabilone is administered orally, by capsules, which is the most common and convenient route of drug delivery. Thus, the major challenge lies in its poor bioavailability because the poor aqueous solubility, the low permeability and the low dissolution rate of poorly water soluble drugs in the aqueous gastrointestinal fluids often lead to low bioavailability.

Various procedures are used for the solubility enhancement of poorly soluble drugs which involve physical and chemical modifications of the drugs and other methods like crystal engineering, particle size reduction, solid dispersion, salt formation, complexation, and so on. (Savjani et al., 2012)

Several studies confirm that cyclodextrins increase the aqueous solubility of THC, the main cannabinoid. (Hippalgaonkar et al., 2011) (Hazekamp et al., 2006)

Therefore, the solubility increase of nabilone, a synthetic cannabinoid of THC, by complexation with various cyclodextrins has been investigated in the present work.

In this study the inclusion complex formation-based technique via cyclodextrins as agents was used by inserting nabilone, the nonpolar guest molecule or the nonpolar region of one molecule, into the cavity of cyclodextrins, RAMEB or HP-β- CD, known as hosts.

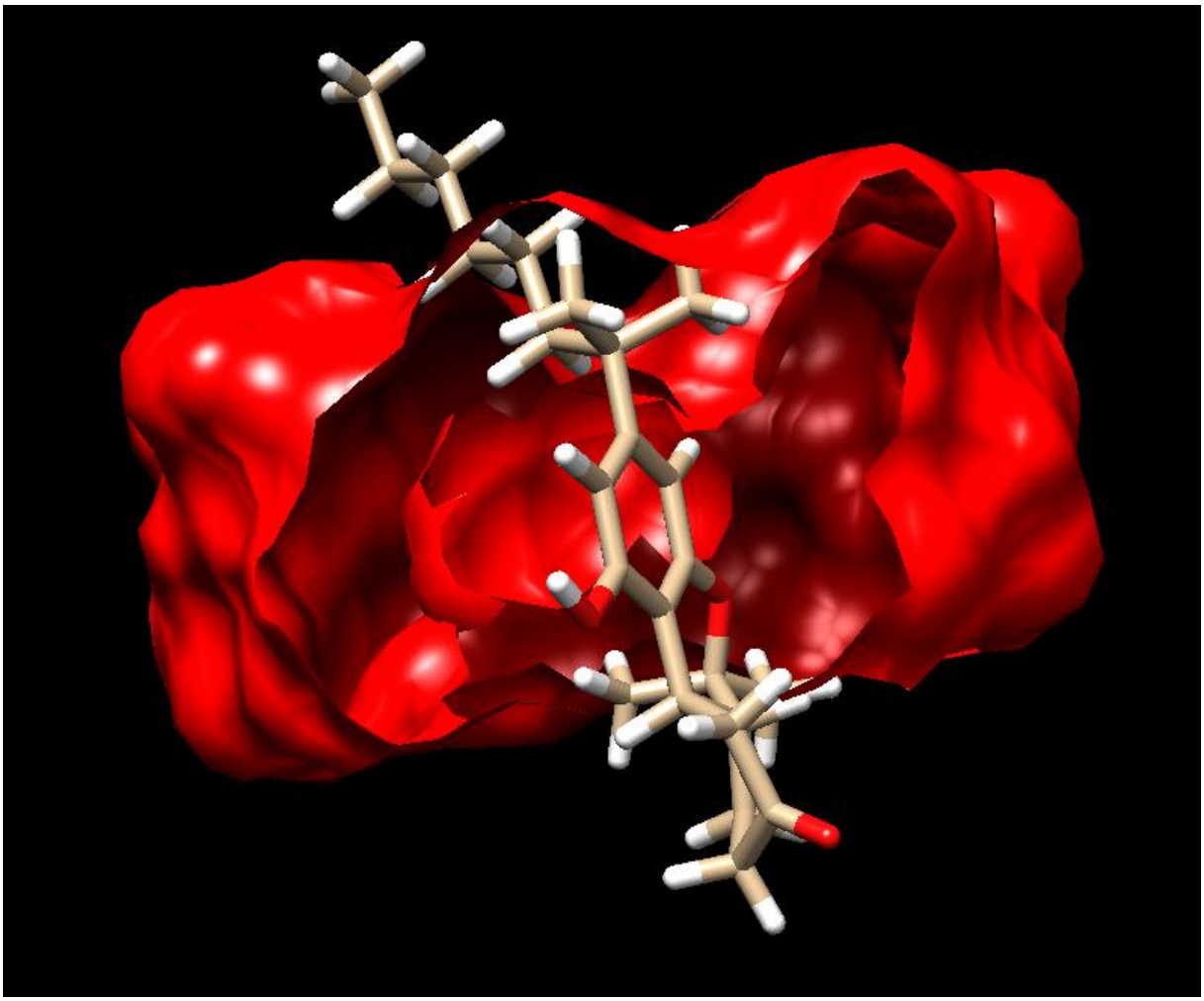


Figure 24: Structure of a nabilone: cyclodextrin (red) complex.

The effect of rising concentration of CDs on aqueous solubility of the agent is usually resolved using phase-solubility studies according to the Higuchi and Connors.

In figure 25 we can see that the A-Type phase-solubility profiles are achieved when the solubility of the therapeutic agent increases with increasing concentration of CDs. AL-type phase-solubility profile is obtained when the complex is first order with respect to CDs, meaning linear increase in solubility of the compound as a function of CDs concentration.

A positive deviation from linearity with increasing concentration of CDs is described as the AP-type phase-solubility profile. In other words, when the complex is first order with respect to the therapeutic agent, but second or higher order with respect to the CDs. (Hazekamp et al., 2006)

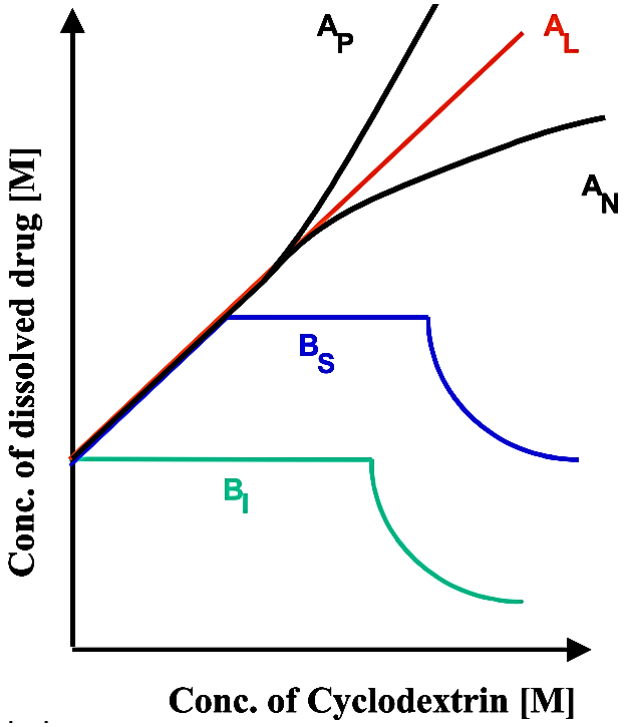


Figure 25: Higuchi and Connors phase-solubility diagram. (Loftsson et al., 2002)

Manila et al. demonstrated that Δ⁹-THC yields an AL-Type phase-solubility curve with HP-β-CD, indicating the formation of 1:1 inclusion complexes between Δ⁹-THC and CD. (Hippalgaonkar et al., 2011)

In this study the nabilone:CD complex showed affinity towards the AP-type as demonstrated in point 5.5 “lyophilization test”, meaning a positive deviation from linearity with increasing concentration of CDs, thus a 1:2 nabilone:CD complex.

This outcome correlates with the results of Hippalgonakar et al. suggesting that Δ^8-THC forms 1:2 inclusion complexes with HP-β-CD and RAMEB. Their reported stability constant ($K1:1=12200M^{-1}$) was less than the values we determined in our study for nabilone (K1:1>16000). Aside from differences in the chemical structure another reason for the observed variations here could be differences in the experimental protocol.

Interestingly, the phenomenon we discovered during the “time depending behavior experiment” left many questions and inspired other tests (filtration and concentration stability test) leading to the conclusion that, according to our data, the concentration of the soluble nabilone:CD complex is only stable after the un-dissolved nabilone was removed from the solution.

As mentioned earlier, the outcome of concentration decrease of soluble nabilone, after it was added additionally to the filtered solution, could be an indication that a stoichiometric change of the complex is happening. Possible formations that can occur in the solutions are 1:1, 1:2 or 2:1 complexes. (Figure 26)

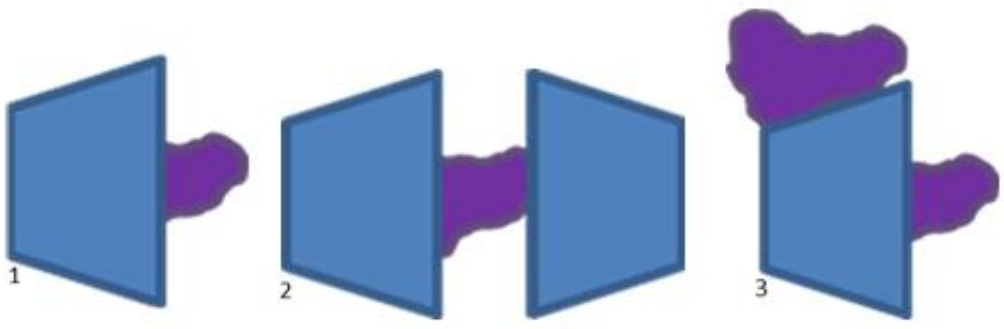


Figure 26: Soluble and less soluble nabilone:CD formations.

Very high solubility:	1) 1:1 complex
Good solubility:	2) 1:2 complex
Poor solubility:	3) 2:1 complex

In case of the 1:1 complexation (complex 1 in figure 26) two different orientations of nabilone in the cavity could be possible. Both orientations lead to different stabilities of the complexes - at thermodynamic equilibrium one orientation dominates. Additionally, another 1:1 complex is possible, where the nabilone is located outside the CD's rim. However, the formation of these complexes is the reason for the tremendous solubility enhancement of nabilone by complexation in aqueous solution.
When one additional cyclodextrin molecule forms a complex with an existing 1:1 formation a 1:2 complexation (complex 2 in figure 26) occurs. Here nabilone serves as a linker connecting two CD molecules by binding in their cavities. It seems reasonable that this formation also has a very positive effect on the solubility since it represents the AP-type phase-solubility profile, as mentioned before.
On the other hand, when one additional nabilone binds outside the CD's rim on an existing 1:1 complex a 2:1 formation (complex 3 in figure 26) results. This arrangement could be a reason for the decrease in solubility but at this point a lower solubility of such an aggregate can be assumed, because the solvent accessible surface of the complex is more hydrophobic. Therefore, it is safe to say that such a 2:1 complex is most unlikely to enhance the solubility.

In conclusion, however, all three association complexes could be the reason for the increase of the overall solubility of nabilone. Complex 3 (Figure 26), is postulated to have higher solubility than nabilone alone, but it is less soluble than the other two complexes. The first two options (1:1 and 1:2) are at first the main reason for increasing solubility. Nonetheless, if the first two options reach their saturation point it could also cause flocculation and thus decreasing solubility.
The fact that nabilone can not only bind in the cavity of one CD but also connect two CD molecules and further bind on the surface of a CD's rim is leading to the assumption that the solutions may contain other formations of higher order which may cause this concentration decrease.
Figure 27 summarizes some of the postulated aggregates.

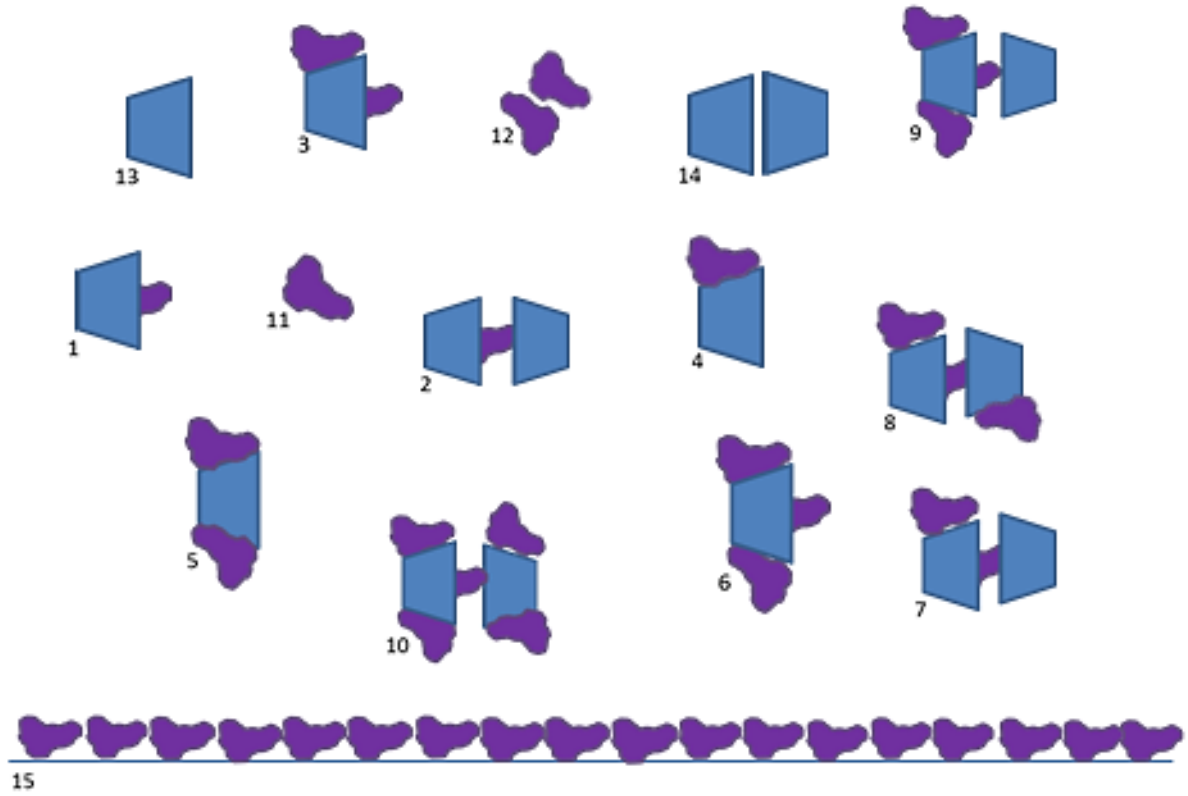


Figure 27: Un-dissolved nabilone and different expected nabilone: CD complexes of higher order with different solubility amounts in equilibrium to each other.

Very high solubility: 1, 13, 14

Good solubility: 2, 4

Poor solubility: 5, 7

Very poor solubility: 3, 6, 8, 9, 10, 11, 12,

Un-dissolved solid nabilone: 15

We already discussed some complexations before (complex 1, 2 and 3).

As mentioned, nabilone has also an affinity to the outer surface of a CD, thus, it seems possible that not only one nabilone molecule but also two can bind on a CD's rim. This complex (complex 5 in Figure 27) is likely to administer the decrease in solubility.

Now, assuming that one additional nabilone molecule can bind in the cavity of a previously formed complex (5, Figure 27) an arrangement of higher order is formed, a 3:1 complexation (6) which could also be a reason for the solutions instability.

Further, if we expect to find one (3) or two (6) additional nabilone molecules on the rim of a 1:1 complex (1), then it is also imaginable to find one (7) or more nabilone molecules on the surface of a 1:2 complex (2).

The first possibility is when two extra nabilone molecules bind on the same CD rim of a previously existing 1:2 formation (9). Secondly, each additional nabilone could also bind on the opposite CD rim, either on the same side or across from each other (8).

According to this thesis up to 4 additional nabilone molecules can bind on a 1:2 complex (10).

It is also necessary to mention that the solution not only contains a very small amount free soluble (11) and un-dissolved nabilone (15) but also maybe aggregates of two (12) or more nabilone molecules.

This assumption can be postulated for the cyclodextrins. It is possible that we can find single CD molecules (13) and maybe even CD dimers (14).

All these discussed formations shown in figure 26 are in equilibrium to each other and manifest at different times due to different equilibrium constants of each complex.

Thus, at this point we cannot exactly tell which one of these complexes specifically is responsible for the concentration decrease.

Figure 28 suggests that the formation of various complexes results also in less soluble complexes. Hence, flocculations are promoted which lead to precipitation of these aggregates, thus to decreasing of the overall concentration of nabilone in the solutions.

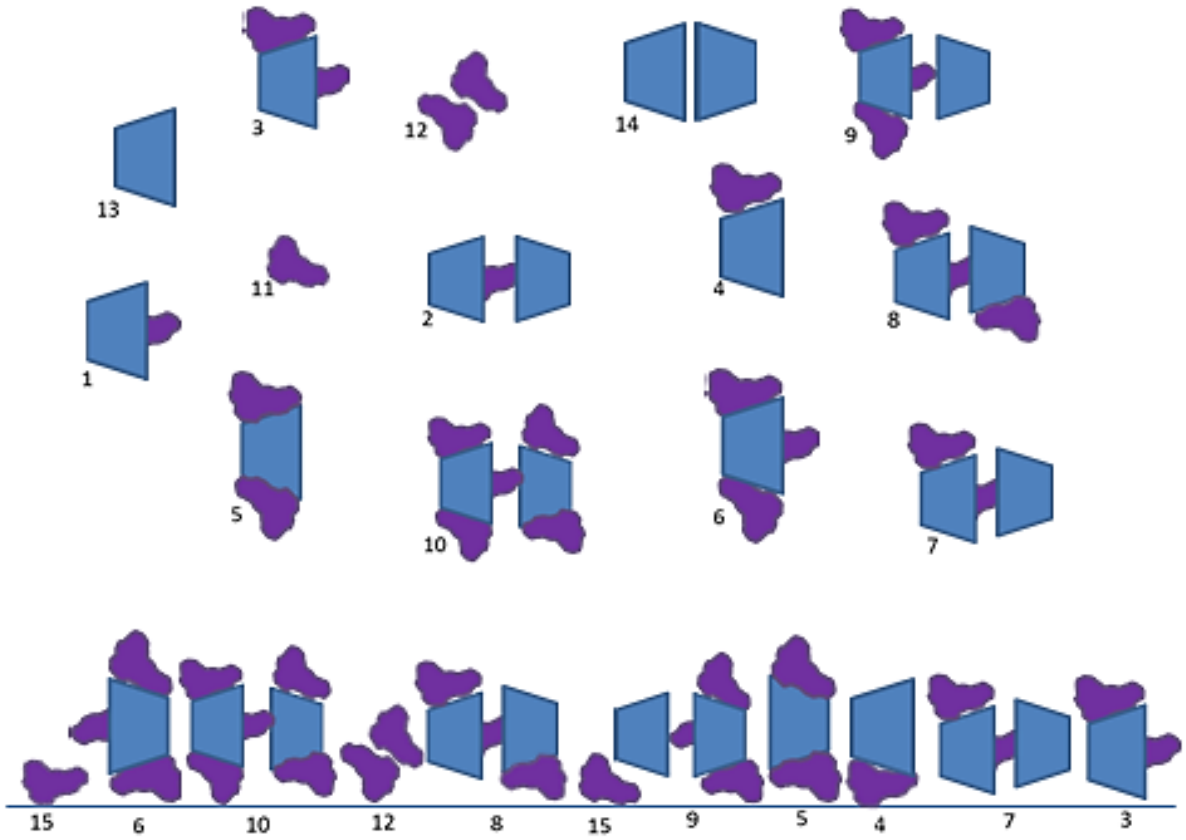


Figure 28: Un-dissolved nabilone and precipitated nabilone: CD complexations in equilibrium to their soluble counterpart.

We assume that the 2:1 complexes (3 and 5 in Figure 27 and Figure 28) and those of higher order (6, 7, 8, 9, and 10) as well as the solid form of nabilone are promoting the overall decrease in concentration.
Since nabilone has a very poor aqueous solubility it seems logical that the more nabilone is binding on a CD, the less soluble the growing complex is going to be.

An indication for this thesis is providing the “filtration and the stability-experiment”.
The fact that by removing un-dissolved nabilone from the solution, the concentration decrease is stopped and reaches a stability point and when afterwards excess amount of nabilone is added to the stable solution the concentration decrease begins again is indicating an important stoichiometric change.
Hence, excess amount of nabilone supports the genesis of these numerous insoluble formations to occur sooner or later, depending on their equilibrium constant and the formulation kinetics.

In case of the cyclodextrins we used HP-β-CD and RAMEB to determine the phase solubility studies. It is evident that RAMEB enhances the solubility of nabilone to a higher extent.

Unfortunately, RAMEB as well as the used HP-β-CD are technical products (see Materials and Methods - chapter 4). Therefore, RAMEB is not an exactly defined compound because its substitution by methyl groups at positions 2 and 6 are rather randomly and consequently RAMEB is a mixture of different substituted compounds. In this regard the use of a pure 2, 6-di-O-methyl-β-cyclodextrin would be favorable considering that the methylation is definite in position 2 and 6, unlike RAMEB where we can find partial substitution at OH of C6, C2 (or C3) of each glucose unit.

In conclusion, the results from this study demonstrate clearly that without the presence of CD's nabilone is practically insoluble in pure water hence, the aqueous solubility can be increased by both cyclodextrins through complexation.
The observed time depending decrease of concentration is most likely associated to the formation of complexes higher order due to the excess amount of nabilone in the solutions. Therefore, a filtration of the nabilone: cyclodextrin solution is an auspicious way to stabilize the concentration for a longer period of time.
Certainly the CD complexation of nabilone is a promising way for producing water based solutions without the need of other solubilizers or organic solvents.
Hopefully the results achieved in this study will be a help for the development of further cyclodextrin studies with nabilone.

8. ANNEX

Raw data:

TIME DEPENDING BEHAVOIR:

The concentration of nabilone in a 10^{-3} molar water/ HP-β-CD mixture after sampling every hour.

Time(h) of stirring	Absorption	nab(mol/l)
1	0,082	0,0000236
2	0,085	0,0000245
3	0,08	0,0000231
4	0,079	0,0000228
5	0,059	0,000017

The concentration of nabilone in a 10^{-3} molar water/ RAMEB mixture after sampling every hour.

Time(h) of stirring	Absorption	nab(mol/l)
1	0,037	0,0000106
2	0,108	0,0000312
3	0,077	0,0000222
4	0,069	0,0000199
5	0,067	0,0000193

FILTRATION EXPERIMENT:

The concentration of nabilone in a 10^{-3} molar RAMEB solution.

After 2 hours of stirring the un-dissolved nabilone was filtrated from the solution and then measured every 50 hours.

Time(h)	Absorption	nab(mol/l)
2	0,059	0,00001705
50	0,062	0,000017919
100	0,062	0,000017919
150	0,061	0,00001763
200	0,061	0,00001763
250	0,061	0,00001763
300	0,061	0,00001763
350	0,061	0,00001763

The concentration of nabilone in a 10^{-3} molar HP-β-CD solution.

After 2 hours of stirring the un-dissolved nabilone was filtrated from the solution and then measured every 50 hours.

Time(h)	Absorption	nab(mol/l)
2	0,051	0,00001474
50	0,055	0,00001589
100	0,056	0,000016184
150	0,056	0,000016184
200	0,055	0,00001589
250	0,055	0,00001589
300	0,055	0,00001589
350	0,055	0,00001589

STABILITY OF CONCENTRATION EXPERIMENT:

The concentration of nabilone in a 10^{-3} molar RAMEB solution.

The solution was stirred for 2 hours, filtrated, then additional nabilone was added and measured every 50 hours.

Time(h)	Absorption	nab(mol/l)
2	0,179	0,00005173
50	0,965	0,000278
100	0,91	0,000263
150	0,864	0,000249
200	0,85	0,00024
250	0,847	0,000244
300	0,84	0,000242
350	0,821	0,000237

The concentration of nabilone in a 10^{-3} molar HP-β-CD solution.

The solution was stirred for 2 hours, filtrated, then additional nabilone was added and measured every 50 hours.

Time(h)	Absorption	nab(mol/l)
2	0,28	0,00008092
50	0,71	0,0002052
100	0,698	0,0002017
150	0,682	0,000197
200	0,65	0,000187
250	0,632	0,000182
300	0,602	0,000173
350	0,591	0,00017

ETHANOLIC SOLUTIONS:

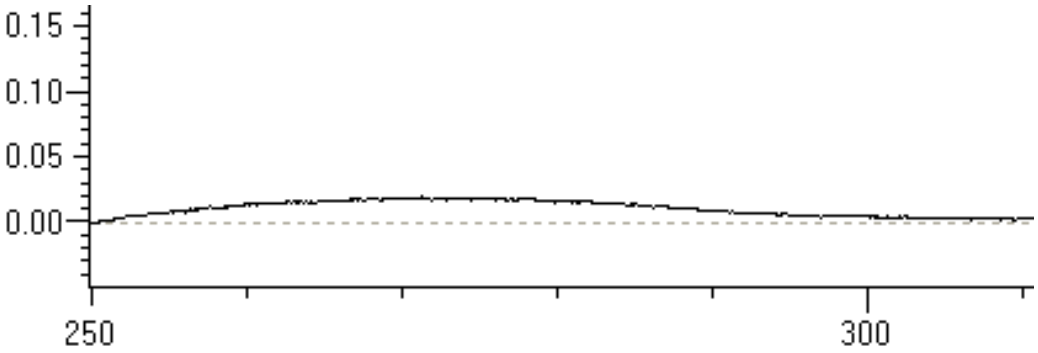
The concentration of nabilone in the presence of water/ ethanol mixtures with an increasing amount of ethanol in the solutions.

EtOH%	Absorption	nab(mol/l)
10	0,007	0,00000202
20	0,016	0,00000462
30	0,047	0,00001358
40	0,061	0,00001763
60	0,063	0,00001820
80	0,091	0,0000263
100	0,173	0,00005

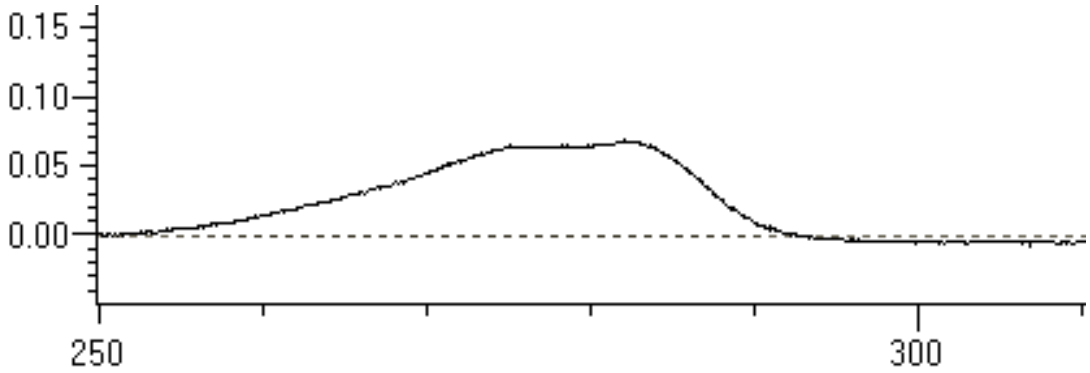
The concentration of nabilone in the same solutions measured again after 3 weeks.

EtOH%	Absorption	nab(mol/l)
80	0,091	0,000022832
60	0,065	0,000018786
40	0,007	0,000002023
30	0	0
20	0	0
10	0	0

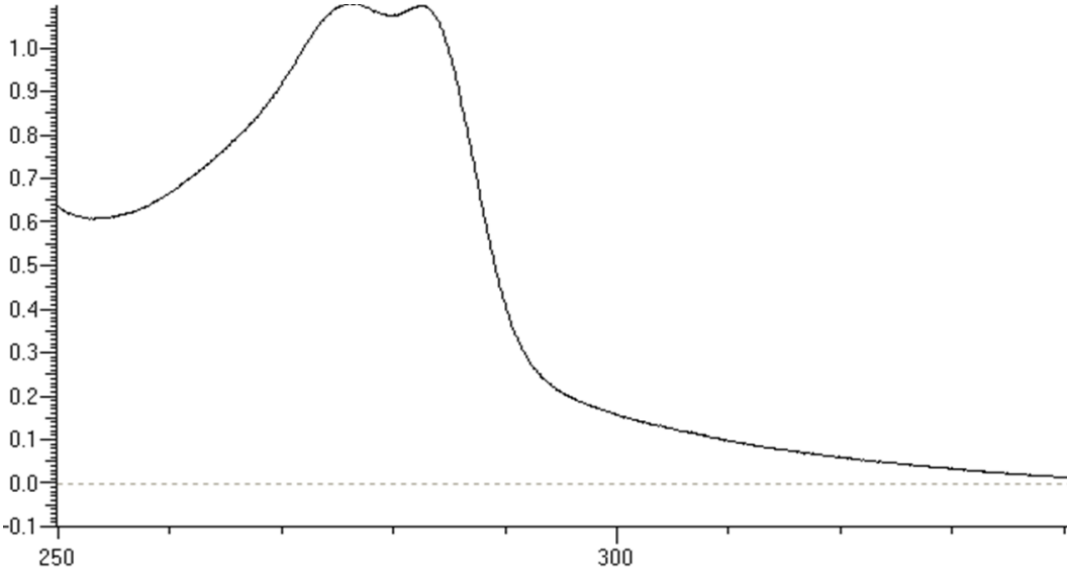
9.1 **UV-Spectra**



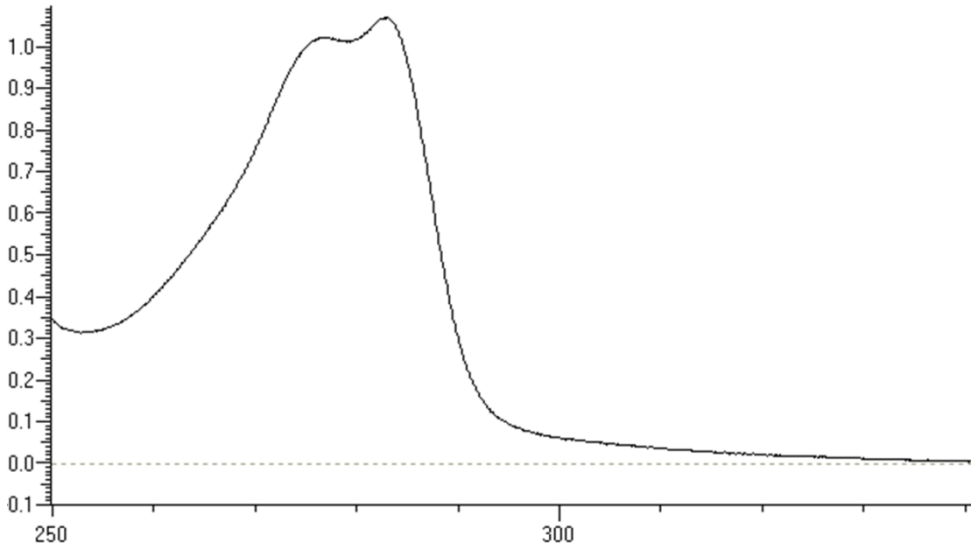
UV spectrum of a nabilone stock solution diluted 1:100 in a 20% ethanolic/ water mixture.



UV spectrum of a nabilone stock solution diluted 1:100 in a 60% ethanolic/ water mixture.



UV spectrum of nabilone in a 10^{-3} molar HP-β-CD solution after lyophilization.



UV spectrum of nabilone in a 10^{-4} molar HP-β-CD solution after lyophilization.

REFERENCE LIST

Aso, E., Ferrer, I. (2014). Cannabinoids for treatment of Alzheimer's disease: moving toward the clinic. Frontiers in Pharmacology, 5, 37. doi: org/10.3389/fphar.2014.00037

Arrabal, S., Lucena, M. A., Canduela, M. J., Ramos-Uriarte, A., Rivera, P., Serrano, A., Suárez, J. (2015). Pharmacological Blockade of Cannabinoid CB1 Receptors in Diet-Induced Obesity Regulates Mitochondrial Dihydrolipoamide Dehydrogenase in Muscle. PLoS one, 10(12), e0145244. doi: org/10.1371/journal.pone.0145244

Bachmeier, C., Beaulieu-Abdelahad, D., Mullan, M., Paris, D. (2013). Role of the cannabinoid system in the transit of beta-amyloid across the blood-brain barrier. Mol Cell Neurosci, 56, 255-262. doi: 10.1016/j.mcn.2013.06.004

Berdyshev, E. V., Boichot, E., Germain, N., Allain, N., Anger, J. P., Lagente, V. (1997). Influence of fatty acid ethanolamides and Δ^9-tetrahydrocannabinol on cytokine and arachidonate release by mononuclear cells. European Journal of Pharmacology, 330(2-3), 231-240. doi:10.1016/S0014-2999(97)01007-8

Bisogno, T., Berrendero, F., Ambrosino, G., Cebeira, M., Ramos, J. A., Fernandez-Ruiz, J. J., Di Marzo, V. (1999). Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. Biochemical and Biophysical Research Communications, 256(2), 377-380. doi:10.1006/bbrc.1999.0254

Broderick Mike. (2013). Basic UV-Vis Theory, Concepts and Applications Basic. ThermoSpectronic, 1–28.

Buczynski, M. W., Parsons, L. H. (2010). Quantification of brain endocannabinoid levels: methods, interpretations and pitfalls. British Journal of Pharmacology, 160(3), 423–442. doi: org/10.1111/j.1476-5381.2010.00787

Burstein, S. H., Zurier, R. B. (2009). Cannabinoids, Endocannabinoids, and Related Analogs in Inflammation. The AAPS Journal, 11(1), 109. doi.org/10.1208/s12248-009-9084-5

Cabral, G. A., Griffin-Thomas, L. (2009). Emerging Role of the CB2 Cannabinoid Receptor in Immune Regulation and Therapeutic Prospects. Expert Reviews in Molecular Medicine, 11, e3. doi: org/10.1017/S1462399409000957

Coffey, R. G., Snella, E., Johnson, K., Pross, S. (1996). Inhibition of macrophage nitric oxide production by tetrahydrocannabinol in vivo and in vitro. Int J Immunopharmacol, 18(12), 749-752. doi:10.1016/S0192-0561(97)85557-9

Cravatt, B. F., Giang, D. K., Mayfield, S. P., Boger, D. L., Lerner, R. A., Gilula, N. B. (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature, 384, 83-87. doi:10.1038/384083a0

Deadwyler, S. A., Hampson, R. E., Mu, J., Whyte, A., Childers, S. (1995). Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. Journal of Pharmacology and Experimental Therapeutics, 273(2), 734-743.

Dinh, T. P., Carpenter, D., Leslie, F. M., Freund, T. F., Katona, I., Sensi, S. L., Piomelli, D. (2002). Brain monoglyceride lipase participating in endocannabinoid inactivation. Proceedings of the National Academy of Sciences of the United States of America, 99(16), 10819–10824. doi: org/10.1073/pnas.152334899

Duncan, M., Galic, M. A., Wang, A., Chambers, A. P., McCafferty, D. M., McKay, D. M., Sharkey, K. A., Pittman, Q. J. (2013). Cannabinoid 1 receptors are critical for the innate immune response to TLR4 stimulation. American Journal of Physiology- Regulatory, Integrative and Comparative Physiology, 305(3), R224-231. doi: 10.1152/ajpregu.00104.2013

Elphick, M. R., Egertová, M. (2001). The neurobiology and evolution of cannabinoid signalling. Philosophical Transactions of the Royal Society of London. Series B, 356(1407), 381–408. doi: org/10.1098/rstb.2000.0787

Esposito, G., Scuderi, C., Valenza, M., Togna, G. I., Latina, V., De Filippis, D., Steardo, L. (2011). Cannabidiol Reduces Aβ-Induced Neuroinflammation and Promotes Hippocampal Neurogenesis through PPARγ Involvement. PLoS One, 6(12), e28668.

Eubanks, L. M., Rogers, C. J., Iv, A. E. B., Koob, G. F., Arthur, J., Dickerson, T. J., Janda, K. D. (2008). A Molecular Link Between the Active Component of Marijuana and Alzheimer's Disease Pathology. NIH Public Access, 3(6), 773–777. doi: 10.1021/mp060066m

Griffin, G., Tao, Q., Abood, M. E. (2000). Cloning and pharmacological characterization of the rat CB(2) cannabinoid receptor. J Pharmacol Exp Ther, 292(3), 886–894.

Hazekamp, A., Verpoorte, R. (2006). Structure elucidation of the tetrahydrocannabinol complex with randomly methylated beta-cyclodextrin. European Journal of Pharmaceutical Sciences, 29(5), 340-7. doi: 10.1016/j.ejps.2006.07.001

Hemmer, B., Cepok, S., Nessler, S., Sommer, N. (2002). Pathogenesis of multiple sclerosis: an update on immunology. Curr Opin Neurol, 15(3), 227-31.

Hippalgaonkar, K., Gul, W., ElSohly, M. A., Repka, M. A., Majumdar, S. (2011). Enhanced Solubility, Stability, and Transcorneal Permeability of Δ^8-Tetrahydrocannabinol in the Presence of Cyclodextrins. AAPS PharmSciTech, 12(2), 723–731. doi: org/10.1208/s12249-011-9639-5

Husni, A. S., Mccurdy, C. R., Radwan, M. M., Ahmed, S. A., Slade, D., Ross, S. A., Cutler, S. J. (2015). Evaluation of Phytocannabinoids from High Potency Cannabis sativausing In Vitro Bioassays to Determine Structure-Activity Relationships for Cannabinoid Receptor 1 and Cannabinoid Receptor 2. NIH Public Access, 23(9), 4295–4300. doi: 10.1007/s00044-014-0972-6

Iring, A., Ruisanchez, E., Leszl-Ishiguro, M., Horváth, B., Benkő, R., Lacza, Z., Benyó, Z. (2013). Role of endocannabinoids and cannabinoid-1 receptors in cerebrocortical blood flow regulation. PloS One, 8(1), e53390. doi: org/10.1371/journal.pone.0053390

Iuvone, T., Esposito, G., Esposito, R., Santamaria, R., Di Rosa, M., Izzo, A. A. (2004). Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa,

on beta-amyloid-induced toxicity in PC12 cells. Journal of Neurochemistry, 89(1), 134–141. doi: 10.1111/j.1471-4159.2003.02327

Jarai, Z., Wagner, J. A., Goparaju, S. K., Wang, L., Razdan, R. K., Sugiura, T., Kunos, G. (2000). Cardiovascular Effects of 2-Arachidonoyl Glycerol in Anesthetized Mice. Hypertension, 35(2), 679–684.

Katona, I., Sperlágh, B., Sík, A., Käfalvi, A., Vizi, E. S., Mackie, K., Freund, T. F. (1999). Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 19(11), 4544–4558.

Kaczocha, M., Glaser, S. T., Maher, T., Clavin, B., Hamilton, J., O'Rourke, J., Thanos, P. K. (2015). Fatty acid binding protein deletion suppresses inflammatory pain through endocannabinoid/N-acylethanolamine-dependent mechanisms. Molecular Pain, 11, 52. doi: org/10.1186/s12990-015-0056-8

Khuntawee, W., Wolschann, P., Rungrotmongkol, T., Wong-Ekkabut, J., Hannongbua, S. (2015). Molecular Dynamics Simulations of the Interaction of Beta Cyclodextrin with a Lipid Bilayer. Journal of Chemical Information and Modeling, 55(9), 1894–1902. doi: 10.1021/acs.jcim.5b00152

Kola, B., Farkas, I., Christ-Crain, M., Wittmann, G., Lolli, F., Amin, F., Korbonits, M. (2008). The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. PloS One, 3(3), e1797. doi: 10.1371/journal.pone.0001797

Lichtman, A. H., Blankman, J. L., Cravatt, B. F. (2010). Endocannabinoid Overload. Molecular Pharmacology, 78(6), 993–995. doi:org/10.1124/mol.110.069427

Loftsson, T., Brewster, M. E. (1996). Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. Journal of Pharmaceutical Sciences, 85(10), 1017–1025. doi:org/10.1021/js950534b

Loftsson, T., Stefánsson, E. (2002). Cyclodextrins in eye drop formulations: enhanced topical delivery of corticosteroids to the eye. Acta Ophthalmologica Scandinavica, 80(2), 144–50. doi: 10.1034/j.1600-0420.2002.800205

Loftsson, T., Jarho, P., Másson, M., Järvinen, T. (2005). Cyclodextrins in drug delivery. Expert Opin Drug Deliv, 2(2), 335-51.

Lowin, T., Straub, R. H. (2015). Cannabinoid-based drugs targeting CB1 and TRPV1, the sympathetic nervous system, and arthritis. Arthritis Research & Therapy, 17(1), 226. doi: org/10.1186/s13075-015-0743

Mackie, K., Hille, B. (1992). Cannabinoids inhibit N-type calcium channels in neuroblastoma - glioma cells. Proceedings of the National Academy of Sciences of the United States of America, 89(9), 3825–3829.

Malfait, A. M., Gallily, R., Sumariwalla, P. F., Malik, A. S., Andreakos, E., Mechoulam, R., Feldmann, M. (2000). The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. Proceedings of the National Academy of Sciences of the United States of America., 97(17), 9561–9566.

Mannila, J., Järvinen, T., Järvinen, K., Tarvainen, M., Jarho, P. (2005). Effects of RM-beta-CD on sublingual bioavailability of Δ^9-tetrahydrocannabinol in rabbits. Eur J Pharm Sci , 26(1), 71-7. doi:10.1016/j.ejps.2005.04.020

Martín-Moreno, A. M., Brera, B., Spuch, C., Carro, E., García-García, L., Delgado, M., de Ceballos, M. L. (2012). Prolonged oral cannabinoid administration prevents neuroinflammation, lowers β-amyloid levels and improves cognitive performance in Tg APP 2576 mice. Journal of Neuroinflammation, 9(1), 8. doi:org/10.1186/1742-2094-9-8

Miranville, A., Herling, A. W., Biemer-Daub, G., Voss, M. D. (2010). Reversal of inflammation-induced impairment of glucose uptake in adipocytes by direct effect of CB1 antagonism on adipose tissue macrophages. Obesity (Silver Spring, Md.), 18(12), 2247–54. doi: 10.1038/oby.2010.81

More, S. V., Choi, D.-K. (2015). Promising cannabinoid-based therapies for Parkinson's disease: motor symptoms to neuroprotection. Molecular Neurodegeneration, 10(1), 17. doi: org/10.1186/s13024-015-0012-0

Munro, S., Thomas, K. L., Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. Nature, 365(6441), 61-5. doi:10.1038/365061a0

Nadler, V., Mechoulam, R., Sokolovsky, M. (1993). The non-psychotropic cannabinoid (+)-(3S,4S)-7-hydroxy- Δ 6- tetrahydrocannabinol 1,1-dimethylheptyl (HU-211) attenuates N-methyl-D-aspartate receptor-mediated neurotoxicity in primary cultures of rat forebrain. Neurosci Lett, 162(1-2), 43-5.

Newton, C. A., Klein, T. W., Friedman, H. (1994). Secondary immunity to Legionella pneumophila and Th1 activity are suppressed by Δ^9-tetrahydrocannabinol injection. Infection and Immunity, 62(9), 4015–4020.

Nireesha, G. R., Divya, L., Sowmya, C., Venkateshan, N., Babu, M. N., Lavakumar, V. (2013). Lyophilization / Freeze Drying - A Review. International Journal of Novel Trends in Pharmaceutical Sciences, 3(4), 87–98.

NIST: National Institute of Standard and Technology http://webbook.nist.gov/cgi/cbook.cgi?ID=C51022710&Mask=200#Mass-Spec

Onnainty, R., Schenfeld, E. M., Quevedo, M. A., Fernández, M. A., Longhi, M. R., Granero, G. E. (2013). Characterization of the hydrochlorothiazide: β-cyclodextrin inclusion complex. Experimental and theoretical methods. J Phys Chem B, 117(1), 206-217. doi: 10.1021/jp311274c

Pacher, P., Kunos, G. (2013). Modulating the endocannabinoid system in human health and disease: successes and failures. The FEBS Journal, 280(9), 1918–1943. doi: org/10.1111/febs.12260

Pertwee, R. G., Gibson, T. M., Stevenson, L. A., Ross, R. A., Banner, W. K., Saha, B., Martin, B. R. (2000). O-1057, a potent water-soluble cannabinoid receptor agonist with antinociceptive properties. British Journal of Pharmacology, 129(8), 1577–1584. doi: org/10.1038/sj.bjp.0703245

Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ^9-tetrahydrocannabinol, cannabidiol and Δ^9-tetrahydrocannabivarin. British Journal of Pharmacology, 153(2), 199–215. doi: org/10.1038/sj.bjp.0707442

Pertwee, R. G. (2007). GPR55: a new member of the cannabinoid receptor clan? British Journal of Pharmacology, 152(7), 984–986. doi: org/10.1038/sj.bjp.0707464

Pertwee, R. G., Gibson, T. M., Stevenson, L. A., Ross, R. A., Banner, W. K., Saha, B., Martin, B. R. (2000). O-1057, a potent water-soluble cannabinoid receptor agonist with antinociceptive properties. British Journal of Pharmacology, 129(8), 1577–1584. doi: org/10.1038/sj.bjp.0703245

www.drugs.com/pro/cesamet.html

PubChem: https://pubchem.ncbi.nlm.nih.gov/compound/Nabilone#section=InChI

Rajeevani, K., Mahalakshmi, K., Uma Maheshwar Rao, V. (2015). Review on lyophilization technique. International Journal of Trends in Pharmacy and Life Sciences, 1(1), 130–140.

Ramirez, B. G. (2005). Prevention of Alzheimer's Disease Pathology by Cannabinoids: Neuroprotection Mediated by Blockade of Microglial Activation. Journal of Neuroscience, 25(8), 1904–1913.

Ramos, J., Cruz, V. L., Martínez-Salazar, J., Campillo, N. E., Páez, J. A. (2011). Phys Chem Chem Phys, 13(9), 3660-8. doi: 10.1039/c0cp01456g

Reza, H., Hossein, M.,Reza, A. Sharifzadeh, M., Ejtemaei-Mehr, S., Razmi, A., Ostad, S. N. (2015). The Neuroprotective Effect of Lithium in cannabinoid Dependence is Mediated through Modulation of Cyclic AMP, ERK1/2 and GSK-3β Phosphorylation in Cerebellar Granular Neurons of Rat. Iranian Journal of Pharmaceutical Research, 14(4), 1123–1135.

Roche, R., Hoareau, L., Bes-Houtmann, S., Gonthier, M. P., Laborde, C., Baron, J. F., Haffaf, Y., Cesari, M., Festy, F. (2006). Presence of the cannabinoid receptors, CB1 and CB2, in human omental and subcutaneous adipocytes. Histochem Cell Biol, 126(2), 177-87.

Royal society of chemistry. (2009). Ultraviolet / Visible Spectroscopy, 68. http://www.rsc.org/learn-chemistry/resource/res00000941/spectroscopy-in-a-suitcase-uv-vis-teacher-resources?cmpid=CMP00001304

Russo, E. B. (2011). Taming THC: potential cannabis synergy and phytocannabinoid terpenoid entourage effects. British Journal of Pharmacology, 163(7), 1344–1364. doi: org/10.1111/j.1476-5381.2011.01238

Ryberg, E., Larsson, N., Sjögren, S., Hjorth, S., Hermansson, N.-O., Leonova, J., Greasley, P. J. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. British Journal of Pharmacology, 152(7), 1092–1101. doi: org/10.1038/sj.bjp.0707460

Savjani, K. T., Gajjar, A. K., & Savjani, J. K. (2012). Drug Solubility: Importance and Enhancement Techniques. International Scholarly Research Network Pharmaceutics, 2012, 195727. doi: org/10.5402/2012/195727

Silvestri, C., Di Marzo, V. (2013). The Endocannabinoid System in Energy Homeostasis and the Etiopathology of Metabolic Disorders. Cell Metabolism, 17(4), 475–490. doi: org/10.1016/j.cmet.2013.03.001

Stefano, G. B., Liu, Y., Goligorsky, M. S. (1996). Cannabinoid receptors are coupled to nitric oxide release in invertebrate immunocytes, microglia, and human monocytes. J Biol Chem, 271(32):19238-19242. doi: 10.1074/jbc.271.32.19238

Stefano, G. B., Salzet, M., Rialas, C. M., Mattocks, D., Fimiani, C., Bilfinger, T. V. (1998). Macrophage behavior associated with acute and chronic exposure to HIV GP120, morphine and anandamide: endothelial implications. Int J Cardiol, 64 Suppl 1:S3-13.

Velasco, G., Hernández-Tiedra, S., Dávila, D., Lorente, M. (2016). The use of cannabinoids as anticancer agents. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *64*, 259–266. doi:10.1016/j.pnpbp.2015.05.010

Verty, A. N. A., Stefanidis, A., McAinch, A. J., Hryciw, D. H., Oldfield, B. (2015). Anti-Obesity Effect of the CB2 Receptor Agonist JWH-015 in Diet-Induced Obese Mice. PLoS one, 10(11), e0140592. doi: org/10.1371/journal.pone.0140592

www.wacker.com/cms/de/products/product/product.jsp?product=8970

Walter, L., Stella, N. (2004). Cannabinoids and neuroinflammation. British Journal of Pharmacology, 141(5), 775–785. doi: org/10.1038/sj.bjp.0705667

Wang, J., Qiu, Z., Wang, Y., Li, L., Guo, X., Pham, D.-T., Prud'homme, R. K. (2016). Supramolecular polymer assembly in aqueous solution arising from cyclodextrin host–guest complexation. Beilstein Journal of Organic Chemistry, 12, 50–72. doi: org/10.3762/bjoc.12.7

Ware, M. A., Daeninck, P., Maida, V. (2008). A review of nabilone in the treatment of chemotherapy-induced nausea and vomiting. Therapeutics and Clinical Risk Management, 4(1), 99–107.

Willoughby, K. A., Moore, S. F., Martin, B. R., Ellis, E. F. (1997). The biodisposition and metabolism of anandamide in mice. The Journal of Pharmacology and Experimental Therapeutics, 282(1), 243–247.

http://www.pterodattilo.com/wordpress/tag/agonisti-cannabinoidi/?lang=en

Wu, J., Bie, B., Yang, H., Xu, J. J., Brown, D. L., Naguib, M. (2013). Activation of the CB2 receptor system reverses amyloid-induced memory deficiency. Neurobiol Aging, 34(3), 791-804. doi: org/10.1016/j.neurobiolaging.2012.06.011

www.drugs.com/pro/cesamet.html

Yang, H., Zhou, J., Lehmann, C. (2015). GPR55 – a putative "type 3" cannabinoid receptor in inflammation. Journal of Basic and Clinical Physiology and Pharmacology, 27(3), 297-302. doi: 10.1515/jbcpp-2015-0080.

Zhu, W., Friedman, H., Klein, T. W. (1998). Δ^9-tetrahydrocannabinol induces apoptosis in macrophages and lymphocytes: involvement of Bcl-2 and caspase-1. The Journal of Pharmacology and Experimental Therapeutics, 286(2), 1103–9.

ABSTRACT

Nabilon ist ein vollsynthetisches Derivat des Δ^9-Tetrahydrocannabinols und weist ähnlich wie das pflanzliche Cannabinoid eine geringe Wasserlöslichkeit auf. Cyclodextrine besitzen die Fähigkeit, Einschlussverbindingen mit apolaren Stoffen zu bilden, um dadurch deren Löslichkeit zu verbessern, was auch zu einer besseren Bioverfügbarkeit der Substanz führt.

Das Ziel dieser Diplomarbeit bestand darin, mit Hilfe von zwei Cyclodextrin-Derivaten, nämlich RAMEB und HP-β-CD, die Löslichkeit von Nabilon in Wasser zu erhöhen.

Alle Experimente zeigten nicht lineare Ap Kurven, welche Komplexbildungen höherer Art, nämlich 2:1, bedeuten und beweisen, dass beide Cyclodextrine zu einer verbesserten Löslichkeit von Nabilon beitragen.

Jedoch konnte man beobachten, dass die Konzentration an gelöstem Nabilon in den Cyclodextrin/Wasser Mischungen nach etwa 2 Stunden, anfing sich zu verringern und es dementsprechend zu einer Niederschlags Bildung kam.

Um diesem Phänomen auf den Grund zu gehen, wurden weitere Experimente vorgenommen.

Eine Strategie war, ungelöstes Nabilon aus der Nabilon:Cyclodextrin Lösung mittels Filtration zu entfernen und zu beobachten, wie sich dies auf die Stabilität der Konzentration auswirkt. Als nächstes wurde observiert, ob zusätzliches, entsprechend überschüssiges Nabilon, das nach erfolgter Filtration nachträglich zugesetzt wurde, einen Einfluss auf die Stabilität der Konzentration hat.

Diese Experimente führten zum Ergebnis, dass eine Filtration von ungebundenem Nabilon aus der Lösung zu einer Stabilisierung der Konzentration beiträgt aber sobald man wieder neues Nabilon nachträglich hinzufügt, beginnt die Konzentration zu sinken und ein Niederschlag wird sichtbar.

Dies führt zu der Annahme, dass sich im Laufe der Zeit viele stochiometrisch verschiedene Komplexe ausbilden, die zu dieser beobachteten Verringerung der Löslichkeit führen.

Aufgrund der Tatsache, dass Nabilon nicht nur im Inneren eines CD Moleküls eingeschlossen werden kann, sondern auch Affinität zu deren Oberfläche besitzt

lässt eine Reihe von Verbindungen höherer Ordnung vermuten, welche in dieser Arbeit postuliert werden.
Es ist sehr wahrscheinlich, dass die Abnahme der Konzentration auf Komplexe höherer Ordnung zurückzuführen ist.
Zum jetzigen Zeitpunkt kann man noch nicht mit Sicherheit sagen, welche dieser Aggregate für die Verringerung der Löslichkeit an Nabilon verantwortlich sind.
Mit Sicheheit kann man aber behaupten, dass Nabilon ohne die Anwesenheit von Cyclodextrinen in Wasser fast unlöslich ist.