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Draft Report

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Comparison of Transdermal Penetration from Different Solvents of Free and Cyclodextrin-Complexed Cannabidiol using the Strat - M Membrane Model

Report 885CBD-STRATM

Prepared for
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1. Introduction & Objective

The objective of this project was to compare the transdermal delivery of free and cyclodextrin-complexed cannabidiol (CBD) (Table I), using the skin-mimicking Strat-M membrane model.

Table I Materials tested.

Test Material	Lot #	SBD Identifier	Reception date	Storage
CBD (neat)	N/A	CBD	July 13, 2017	RT
CBD Complex 30%	W7P-KA-0611A	CCD-CBD		
Cyclodextrin	N/A	CCD		

2. Materials & Methods

Test Materials

The transdermal penetration of CBD and its cyclodextrin (CCD) – complexed form (CCD-CBD) was studied in two series of experiments using 4mg/ml stock solutions in DMSO. For the first series of experiments, CDB, CCD and CCD-CBD stock solutions were further dissolved in DMSO:phosphate-buffered saline (PBS) (1:3) at 250 µg/ml, according to the instructions for achieving the maximal solubility of CBD, from Cayman Chemical, Ann Arbor, MI (see highlighted text in the Appendix). For the second round of experiments, stock solutions were further dissolved in caprylic/capric triglycerides (MTC; CAS: 73398-61-5) at 250 µg/ml for CBD and at 800 µg/ml for CCD-CBD Complex (which contains only 30% of the CBD w:w). The standard curve was established and measurements of CBD transdermal penetration were performed using absorbance at 280 nm with Nanodrop spectrophotometer (Thermofisher Scientific, Waltham, MA).

Transdermal penetration model

Strat-M membrane from Sigma (cat. SKBM02560A) was used as the model for quantifying the transdermal penetration of CBD. Strat-M human skin-approximating membrane has been specifically developed by Millipore for studies of transdermal penetration. It is composed of a water-repellent thin waxy layer mimicking the epithelial stratum corneum and an underlying fibrous layer resembling the dermis.

The method for the assessment of the transdermal penetration of CBD and CBD Complex consisted in applying 25 µl of the test materials on top of Strat-M filters and measuring the permeated CBD or CBD Complex (CCD-CBD) in the receiving compartment (50 µl in a humidified chamber) for up to 60 min., using absorbance at 280 nm as a readout.

3. Results and Discussion

Table II shows the absorbance at 280nm of cyclodextrin, CBD and CCD-CBD in a DMSO:PBS (1:3) solution.

Test Material	A280	SEM
Cyclodextrin	0	0.000
CBD	0.415	0.016
CBD Complex 30%	0.197	0.011

Table II. Absorbance at 280nm of three test materials at 250µg/ml dissolved in DMSO:PBS (1:3 v:v).

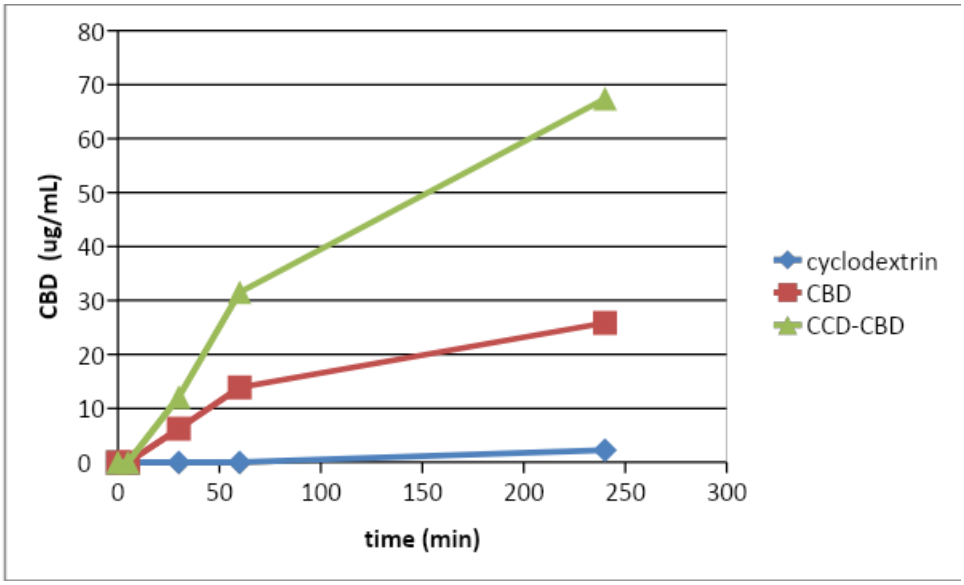


Figure 1A presents the standard curve of correspondence between pure CBD concentrations and optical density measured at 280 nm. It shows that CBD in a solution can be quantified by measuring the absorption at the wavelength of 280 nm. Importantly, cyclodextrin does not show any absorption at that wavelength.

Figure 1B shows that CBD complexed with cyclodextrin (CCD-CBD) in the DMSO:PBS, aqueous-like solution penetrated skin roughly twice as fast as the free CBD, over the period of up at least 3 h. The transmembrane penetration of CCD-CBD formulated in medium chain tryglicerides (MTC) was over 50 times better than the penetration of free CBD dissolved in MTC (Fig. 1C).

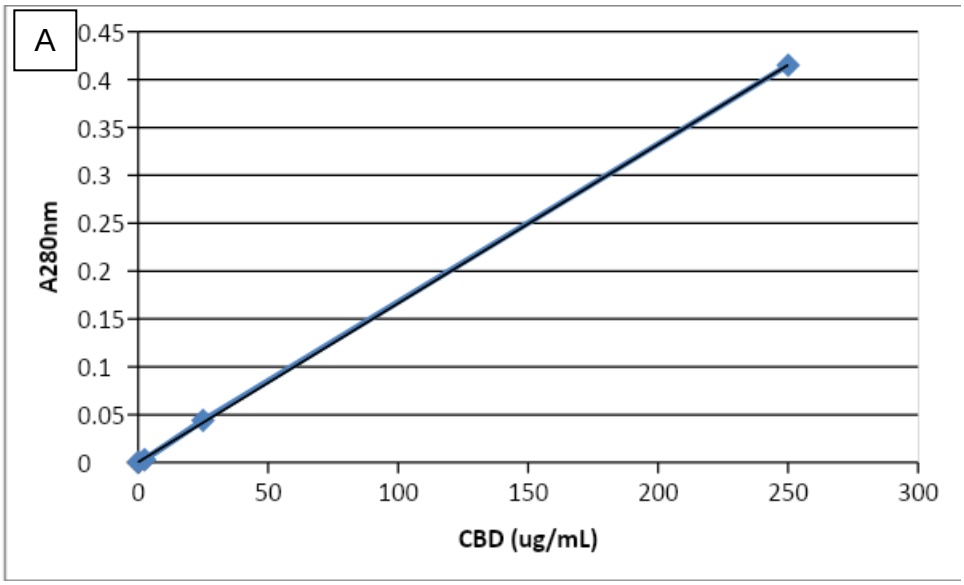
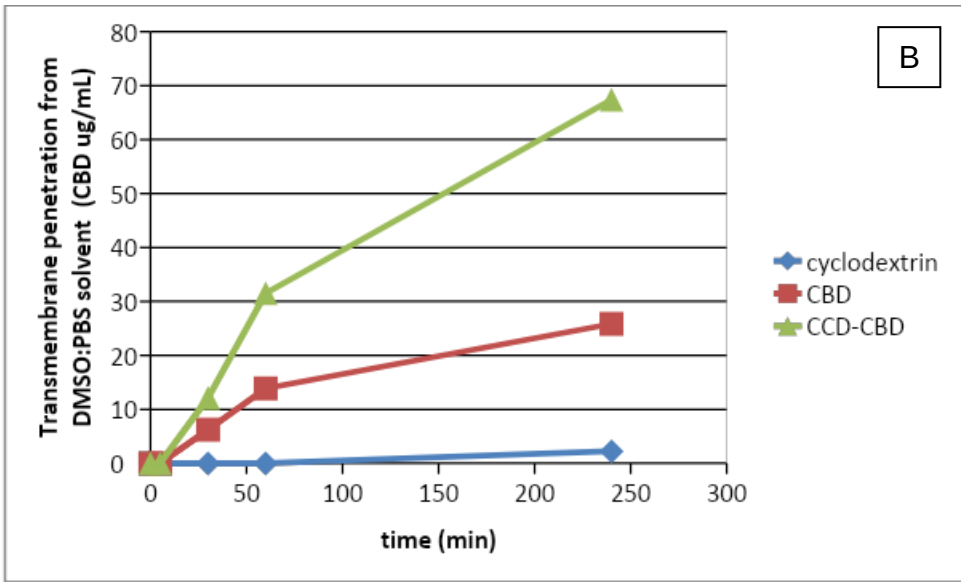
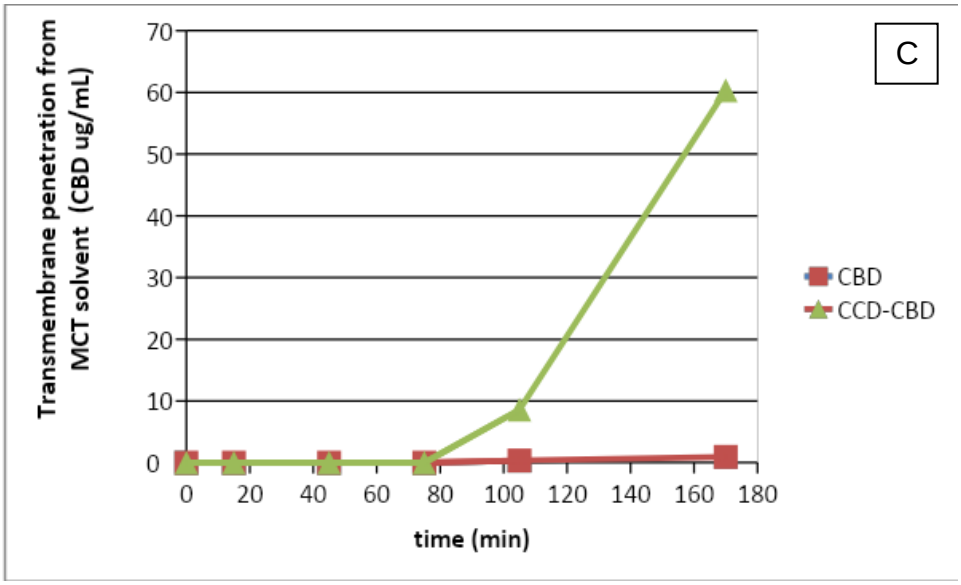


Figure 1. A: Standard curve of correspondence between CBD concentrations and optical density (absorbance) measured at 280 nm.; B: Transmembrane penetration from (DMSO:PBS 1:3 v:v) solvent solutions of pure CBD and CBD formulated in cyclodextrin (CCD-CBD) as a function of time; C: Transmembrane penetration from MTC solvent solutions of pure CBD and CBD formulated in cyclodextrin (CCD-CBD) as a function of time.





4. Conclusion

Based on the results obtained in this series of experiments, it is predicted that complexing CBD with cyclodextrin may significantly improve the transdermal penetration of this cannabinoid.

Experiments performed and Report prepared by:

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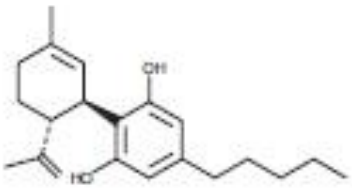
Appendix

PRODUCT INFORMATION

Cannabidiol (exempt preparation)
Item No. 90081

CAS Registry No.:	13956-29-1
Formal Name:	2-[1R-3-methyl-6R-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol
Synonym:	CBD
MF:	$C_{21}H_{30}O_2$
FW:	314.5
Purity:	≥99%
Stability:	≥1 year at -20°C
Supplied as:	A solution in methanol
UV/Vis.:	λ_{max}: 209, 275 nm



Laboratory Procedures

For long term storage, we suggest that cannabidiol (exempt preparation) be stored as supplied at -20°C. It should be stable for at least one year.

Cannabidiol (exempt preparation) is supplied as a solution in methanol. To change the solvent, simply evaporate the methanol under a gentle stream of nitrogen and immediately add the solvent of choice. Solvents such as ethanol, methanol, DMSO, and dimethyl formamide purged with an inert gas can be used. The solubility of cannabidiol (exempt preparation) in these solvents is approximately 35, 30, 60, and 50 mg/ml, respectively.

Cannabidiol (exempt preparation) is sparingly soluble in aqueous buffers. For maximum solubility in aqueous buffers, cannabidiol (exempt preparation) should first be dissolved in DMSO and then diluted with the aqueous buffer of choice. Cannabidiol (exempt preparation) has a solubility of 250 µg/ml in a 1:3 solution of DMSO:PBS (pH 7.2) using this method. We do not recommend storing the aqueous solution for more than one day.

Description

Cannabidiol is an active phytocannabinoid identified in *Cannabis* (composes ~40% of the plant's extract). Unlike Δ^9-THC (Item No. 12068), cannabidiol is considered to be non-psychoactive.[1] Cannabidiol has a very low affinity for CB_1 and CB_2 receptors but acts as an indirect antagonist and is thought to potentiate the effects of Δ^9-THC.[2] Cannabidiol is reported to act as a CB_2 receptor inverse agonist, GPR55 antagonist, and a 5-HT_{1A} receptor agonist.[2-5] It can allosterically modulate µ- and δ-opioid receptors as well as agonize PPARγ receptors and stimulate intracellular calcium release.[6,7]

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