Determination of the Color-Changing Product in Cannabidiol and Propylene Glycol Solutions

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Abstract

Propylene glycol and cannabidiol (CBD) solutions change color overtime at shelf conditions. The main goal of this project was to test CBD and propylene glycol solutions made at concentrations specified by Essential Depot's most popular products during different points in their aging processes in order to figure out what compound is responsible for color change and how strongly color change is dependent on the amount of this product. Methods and techniques developed in this project can help pave the way for other projects, whose goal is to find the color-changing product of vegetable oil-based CBD solutions.

Introduction

CBD products are increasing in popularity because of their widespread legalization and potential to treat anxiety and other disorders[1]; therefore, gaining an understanding of CBD products and how they behave on the shelf has become more and more important in recent years. Some of the most common CBD products are CBD propylene glycol solutions because they can be easily vaporized in comparison to oil-based CBD solutions.

One source of concern for many consumers of propylene glycol CBD solutions is that these solutions tend to change color over time. When uncolored CBD is added to propylene glycol solutions and aged at shelf conditions, the solution turns a reddish-brown color over time (fig. 1). The main goal of this project was to determine what compound is causing this color change and to quantify how much of this color change is associated with what amount of color-changing product. Another goal of this project was to establish methods for determining color-changing products in CBD hemp seed oil and CBD almond sweet oil solutions.

Since Essential Depot sponsors Hampden-Sydney College's chemistry
lab, new propylene glycol and CBD solutions were made to their standards
and tested regularly. To quantify the amount of color-changing product, GC-MS was used, and to quantify color change, UV-Visible light spectrophotometry was used. Structural information about the color-changing

Fig 1: Propylene Glycol (left) vs. Aged Propylene Glycol CBD solution (right)

product was determined using nuclear magnetic resonance (NMR) methods.

In order to establish a procedure to accomplish the same goal using oil-based CBD solutions, liquid chromatography-mass spectrometry (LC-MS) procedures were developed for CBD vegetable oil solutions using Essential Depot products.

There has been some preliminary research into the color-changing product in certain CBD solutions, namely the Beam Test. The Beam Test is a

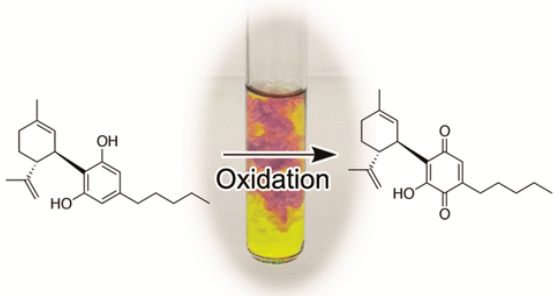

Fig 2: Beam test: Oxidation of CBD in methanolic KOH to form CBDQ[2]

field test, used mainly by law enforcement, which involves the addition of methanolic KOH to CBD solutions leading to the production of cannabidiol quinone (CBDQ), which turns the solution a reddish-purple color[2] (fig. 2).

One compound that is referenced throughout this project is cannabielsoin (CBE). CBE is a relatively newly discovered cannabinoid, only having been discovered in 1988 when its presence was found in rodents[3]. CBE's mechanism of formation in the body was also postulated in the same research group approximately one year later[4] (fig. 3).

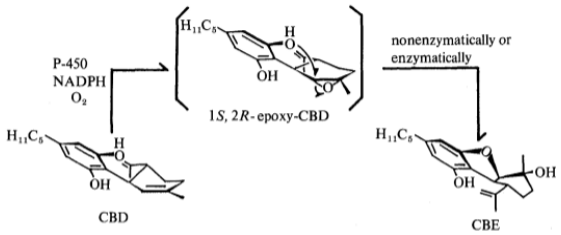

Fig. 3: Mechanism of CBE formation in the body[4]

It has since been determined that CBE can also form from the photo-oxidation of CBD or CBDA.[5] CBE has a different mode of inhibition of CYP1A1-Mediated 7-ethoxyresorufin O-deethylase activity, favoring mixed inhibition as opposed to competitive inhibition favored by CBD. CYP1A1 is a receptor utilized by the drugs granisetron and dacarbazine[6]. All of this is to say that it is important to understand the amounts of CBE in solutions because it has different effects on some key receptors in the body as does CBD.

Methods

The first step of this project was to make CBD propylene glycol solutions in accordance with Essential Depot's product specifications, 33.33 mg of CBD per 1 mL propylene glycol. This goal was accomplished using an analytical balance to weigh out the CBD, combining the CBD with 10 mL of propylene glycol in a 10 mL volumetric flask, and adding the appropriate amount of additional propylene glycol in order to correct for any discrepancies that occurred in weighing exactly 333.3 mg of CBD.

Weekly GC-MS tests were done by adding 50 uL of the freshly prepared CBD propylene glycol solution to a glass GC-MS screw cap vial, adding 950 uL of methanol to the same GC-MS vial, then running the vial through the GC-MS. A specially designed method file was used that only started recording data after the methanol and propylene glycol had gone through the GC column so that only cannabinoid peaks would appear on the spectrum. This same procedure was used in order to run aged propylene glycol solutions through the GC-MS to use as a reference to compare to the weekly trials.

Regular UV-Vis tests were run on the freshly prepared sample and aged reference sample by diluting the samples in propylene glycol ten -fold then using the UV-Vis spectrophotometer to produce a spectrum from 135 nm through 850 nm for each sample at ultra-slow scan speed to ensure appropriate resolution.

LC-MS was run on the hemp seed oil by loosely following a procedure developed by one research group that allowed them to find Glycidyl Esters of Fatty Acids in vegetable oils[7]. First, the solution was diluted in acetone, then it was run through the LC-MS using 99.9% water and 0.1% trifluoroacetic acid as solution A and 90% acetonitrile and 10% water as solution B.

Proton (H1) and Carbon (C13) NMR were run on the cannabinoids in the aged CBD propylene glycol solution at two points in this project. The first sample was prepared by using a separatory funnel with ethyl acetate and water. All of the cannabinoids were trapped in the organic ethyl acetate layer, leaving the propylene glycol in the polar water layer. The ethyl acetate was rotovapped out of the sample, then deuterated chloroform was added to the recovery flask and stirred. Lastly, the sample was run through the NMR.

Thin-layer chromatography was done on the separated cannabinoids from old propylene glycol in a 4:1 mixture of ethyl acetate and hexane in order to determine the solvent's fitness for running a silica column.

The same funnel separation procedure was used again in the preparation of column chromatography of the aged CBD propylene glycol solution. The sample was rotovapped then dissolved in a mixture of 4:1 ethyl acetate and hexane before silica powder was added to the solution. The paste-like solution was rotovapped to get rid of the solvent leaving only the sample in silica powder. This sample was run through a column containing silica gel made with ethyl acetate as a solvent. 11 separate cuts were obtained from this column. GC-MS was run on every cut in the column.

After GC-MS testing, the brightest colored cuts obtained from the first column were combined and run through a second column. The second column was run using a 4:1 mixture of ethyl acetate and hexane as a solvent rather than ethyl acetate in order to ensure the separation of the color-changing product. 10 cuts were obtained from the second column. GC-MS and UV-Vis were run on the cuts in 4:1 ethyl acetate and hexane.

The second run of H1 and C13 NMR was run on the cuts obtained from the second column that had the highest concentrations of a close analog of CBE detected in GC-MS spectra (CBE2). The solvent was rotovapped off in a recovery flask. The dry sample was then dissolved in the recovery flask in deuterated chloroform before ultimately being run through NMR.

Standard solutions of CBE and CBDQ were tested using UV-Vis and GC-MS methods in order to use as a comparison to data obtained from weekly fresh sample testing and column cut testing. The same method file as the weekly tests was used to run the

standards through GC-MS. The same scanning parameters of the regular UV-Vis tests were also used in the testing of the standard solutions.

Results & Discussion

First, a preliminary GC-MS run was done on an aged sample of a CBD propylene glycol solution (fig. 4).

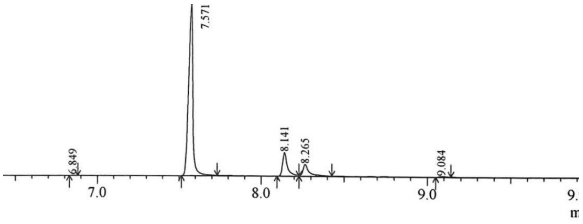

Fig. 4: GC-MS spectrum of Old propylene glycol CBD solution

This spectrum showed that CBD came out at 7.57 minutes retention time (inside the column), two things that were recognized by the GC-MS software as CBE came out at 8.14 minutes retention time and 8.27 minutes retention time. The fact that the two peaks came out at such close retention time and that they both had mass spectra recognized as CBE, showed that they were very close in chemical structure. The first peak is CBE because it came out with the same retention time as did the CBE standard and the second peak will be referenced as CBE2 because it has a very similar structure to CBE.

In the weeks following the preparation of the fresh CBD propylene glycol solution, a relationship was established between time and amount of CBE and CBE2. This curve flattened out around week 7(fig. 5).

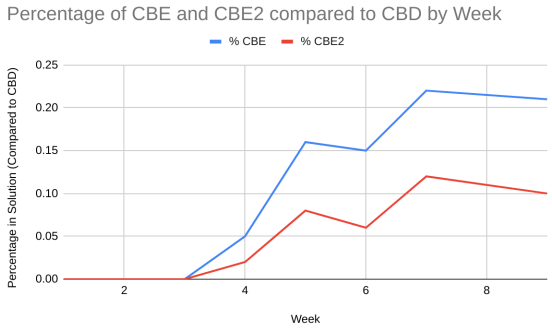

Fig. 5: CBE and CBE2 percentage compared to CBD by week

It should be recognized that CBE and CBE2 stayed approximately the same in their ratio each week. In weeks one through three this graph indicates no CBE or CBE were detected, but there was likely to be trace amounts of CBE and CBE2 hidden in the noise of the GC-MS spectrum. Fragments typical of CBE[8] can be amplified over the spectrum to show that CBE was in fact growing between weeks one and two and two and three (Fig. 6 and Fig. 7). These fragments were not present in notable amounts in the week 1 sample.

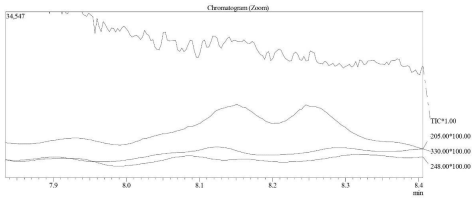

Fig. 6: GC-MS spectrum of week 2 testing with CBE fragments graphed

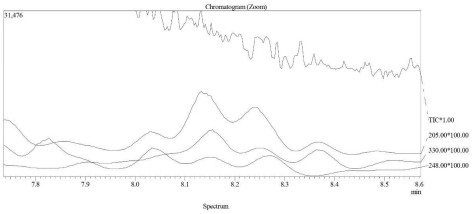

Fig. 7: GC-MS spectrum of week 3 testing with CBE fragments graphed

This data indicates that CBE and CBE2 were in fact growing during weeks one through three because fragments increased and separation got clearer between CBE and CBE2. This observation further proves CBE and CBE2 grew in the same ratio week by week. The fact that CBE and CBE2 grow at the same ratio is important because it indicates that the two are both formed directly from CBD and not one another. CBDQ was not confirmed to be present in any GC-MS spectrum from testing of the freshly prepared or aged propylene glycol CBD solutions.

UV-Vis results for the freshly prepared propylene glycol CBD solution were much more

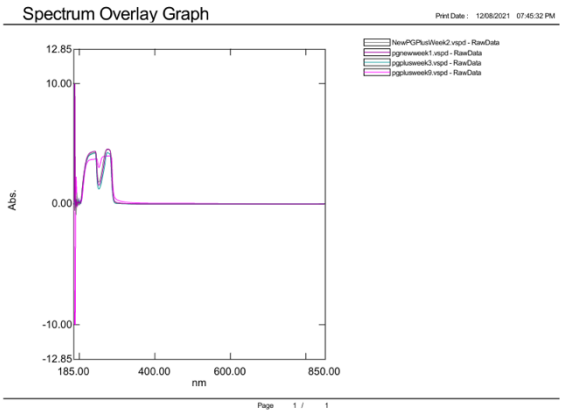

Fig 8: Weekly UV-Vis results

unclear. The peaks at 225 nm and 275 nm wavelength both appeared to go down week by week. In part, this is due to the fact that the week 9 peak overloaded the

spectrophotometer because its peak (pink) goes completely flat at the wavelengths that it is expected to have a maximum value. The tailing of the 275 nm peak is the most significant part in determining the color change and it is clear that week 9 had the most tailing, which gave it its yellowish-red color. Ultimately, however, there were not enough data points to show any strong trend in this data.

More time would likely show that the tailing and absorbance associated with the 275 nm peak increases with time and causes significant color change. The aged CBD propylene glycol solution showed the highest absorbance in this UV region (Fig. 9).

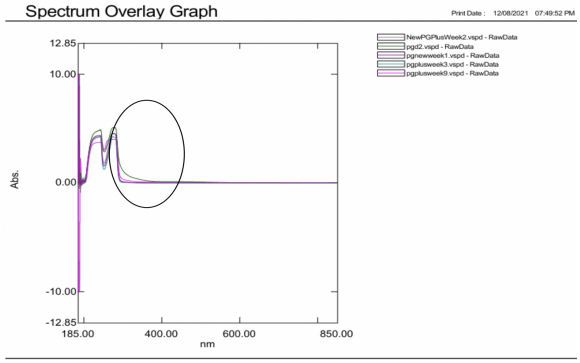

Fig. 9: Aged CBD propylene glycol solution (green) versus weekly tests of fresh CBD propylene glycol solution, the region likely responsible for color change is circled

Column chromatography data gave more conclusive information because it provided more significantly different ratios of CBD compared to CBE and CBE2. Data indicated that in cuts that had higher concentrations of CBE and CBE2 the absorbance of the 274 nm peak was higher (fig. 10). In this data, none of these peaks flattened out the same way that the week 9 result of the fresh CBD propylene glycol solution did, indicating this data can be interpreted as leading directly to color change.

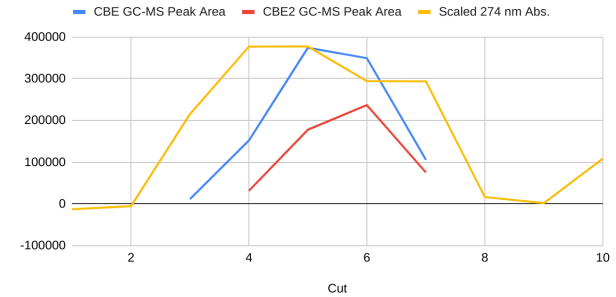

Fig 10: CBE and CBE2 GC-MS peak area versus scaled 274 nm absorbance

The three strongest colored cuts produced from this column were 4, 5, and 6. This lines up with the UV-Vis data, which indicates that these cuts had the highest absorbance at 274 nm. There was not much change in the ratio of CBE and CBE2 to each other. This occurrence is likely due to the fact that the two molecules are so closely related in their structures that it is very difficult to separate them via a standard silica column. One thing that can be concluded, however, is that CBE is uncolored, which is known because the concentrated CBE standard solution was uncolored. This would leave one to conclude that CBE2 is a color-changing product, but CBDQ must also be considered also. GC-MS data did not show evidence of CBDQ, but UV-Vis data showed the presence of CBDQ in only cut 7. CBDQ has a unique peak around 300 nm which CBE and CBD do not have (fig. 11). This peak appeared in cut 7 (fig. 12).

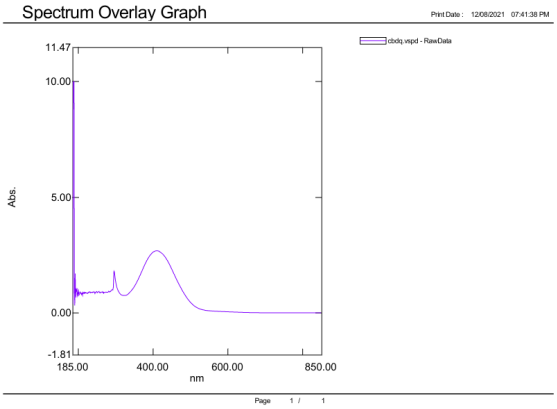

Fig 11: Standard of CBDQ's UV-Vis Spectrum

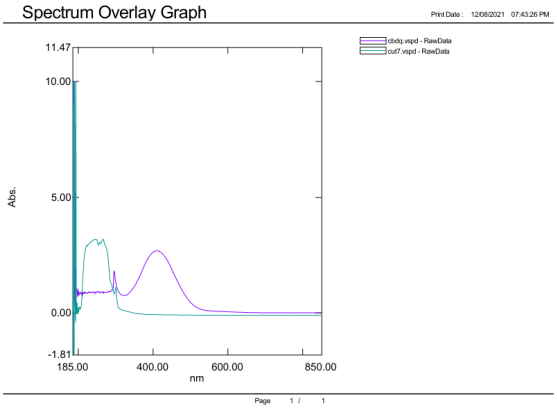

Fig 12: Cut 7's and CBDQ Standard's spectra overlaid

This occurrence was strange because CBDQ itself is brightly colored yellow, which could indicate that the cut with the highest concentration of CBDQ would be the strongest color, but this is not the case. Cut 7 was not visually the strongest colored cut and its spectrum did not have the highest absorbance at 274 nm or absorbance going up to 400 nm. This being said, this data is enough to indicate that CBDQ was not present in high enough amounts to be the main cause of color change in CBD propylene glycol solutions over time at shelf conditions.

Structural data of CBE2 was determined through NMR. The initial NMR contained propylene glycol, which made the CBE and CBE2 peaks practically invisible in comparison, which left only NMR data produced from the column cuts that had the most CBE2. Only H1 NMR was available for this sample despite attempts at running a large number of C13 NMR scans. The H1 NMR clearly indicated that the alcohol group of the left-most ring of CBE (fig. 13) was conserved in the structure of CBE2, while the alcohol on the right-most ring of CBE differed in CBE2 because it was on one of the carbons in the center of the 5 carbon chain (fig. 14). It is not clear which carbon group the alcohol group was moved onto, all that is clear is that it is on carbon next to two other carbons on the chain with two hydrogens each.

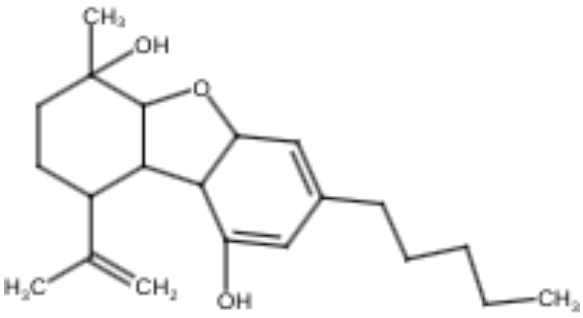

Fig 13: CBE Molecule

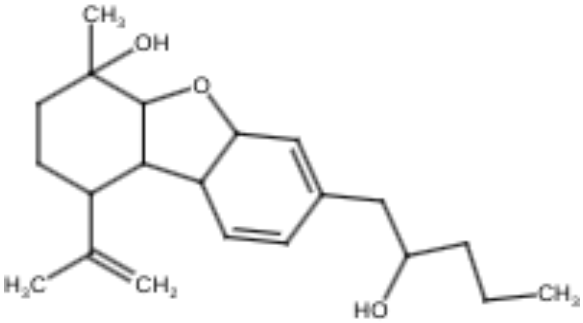

Fig 14: Proposed structure of CBE2 molecule (right-most alcohol could be moved one or two carbons to the right on the carbon chain and be an equally plausible structure)

LC-MS was successful, but not fully. A full spectrum was obtained that very clearly contained CBD. It even appeared that CBE might have been present in the spectrum based on MS data, but the injection size was too high when the sample was run, so there was not any UV-Vis data available to identify the oil. In any case, the procedure would fully work with a decrease in the injection size. For the purposes of this project, it is enough to have gotten an LC-MS spectrum for the CBD oil solution because it shows that it can be done and that other projects going forward can be based around LC-MS.

Conclusion

Data from this project indicated that CBE2 was directly responsible for color change and that color change varied strongly with the amount of CBE2. The three structural possibilities that CBE2 could be have not been thoroughly researched.

Data in this project also indicated that CBDQ was present in CBD propylene glycol solutions, but it was unclear if it increased over time. Either way, CBDQ was not present in high enough amounts to cause color change or even be detectable in propylene glycol CBD solutions run through the GC-MS.

LC-MS procedures were successfully developed for running oil-based CBD solutions. Future research projects can use LC-MS paired with UV-Vis to accomplish similar goals to those accomplished by this project, but with oil-based CBD solutions.

Other than testing oil-based CBD solutions, there are some directions for future research that more directly relate to this project. COSY or two-dimensional H1 NMR can be used to determine the location of the alcohol group on CBE2, this would be the logical next step for this project. Additionally, freshly prepared CBD and propylene glycol solutions could be held in different light environments (dark and light) and tested regularly using UV-Vis and GC-MS. Results from this experiment could give the dependence on CBE amount as a function of light and more importantly give information on if CBE2 is a photo-oxidation product of CBD as is CBE[5] and give different CBE and CBE2 ratios over time and how they relate to color change.

REFERENCES

1. Shannon, S.; Lewis, N.; Lee, H.; Hughes, S. Cannabidiol in Anxiety and Sleep: A Large Case Series. The Permanente journal **2019,** *23*, 18–041. DOI: 10.7812/TPP/18-041.

2. Caprioglio, Deigo; Mattoteia, Daiana; Pollastro, Federica; Negri, Roberto; Lopatriello, Annalisa; Chianese, Giuseppina; Minassi, Alberto; Collado, Juan A.; Munoz, Eduardo; Taglialatela-Scafati, Orazio; Appendino, Giovanni.The Oxidation of Phytocannabinoids to Cannabinoquinoids. J. Nat. Prod. **2020**, *83*(5), 1711-1715. DOI: 10.1021/acs.jnatprod.9b01284

3. Yamamoto, I.; Gohda, H.; Narimatsu, S.; Yoshimura, H. Identification of cannabielsoin, a new metabolite of cannabidiol formed by guinea-pig hepatic microsomal enzymes, and its pharmacological activity in mice. Journal of pharmacobio-dynamics, **2020** *11*(12), 833–838.

4. Yamamoto, Ikuo; Godha, Hiroshi; Narimatsu, Shizuo; Yoshimura, Hidetoshi. Mechanism of Biological Formation of Cannabielsoin from Cannabidiol in the Guinea-Pig, Mouse, Rat, and Rabbit. J. Pharmacobio-Dyn. **1989**, (12), 488-494.

5. Morales, Paula; Hurst, Dow P.; Regio, Patricia H.Molecular Targets of the Phytocannabinoids-A Complex Picture. Prog Chem Org Nat. Prod **2017**, *103*, 103-131. DOI: 10.1007/978-3-319-45541-9_4.

6. Yamaori, Satoshi; Okushima, Yoshimi; Masuda, Kazufumi; Kushihara, Mika; Katsu, Takashi; Narimatsu, Shizuo; Yamamoto, Ikuo; Watanabe, Kazuhito. Structural Requirements for Potent Direct Inhibition of Human Cytochrome P450 1A1 by Cannabidiol: Role of Pentylresorcinol Moiety. Biol. Pharm. Bull **2013**, *36*(7), 1197-1203.

7. Blumhorst, Michael; Venkitasubramanian, Padmesh; Collison, Mark. Direct Determination of Glycidyl Esters of Fatty Acids in Vegetable Oils by LC–MS. *J Am Oil Chem Soc.* **2011**, *88*, 1275-1283. DOI: 10.1007/s11746-011-1873-1.

8. Kuppers, F. J. E. M.; Bercht, C. A. L; Salmink, C. A; Lousberg, R. J. J. CH.; Terlouw, J. K.; Heerma, W. XIV*. Pyrolysis of cannabidiol-analysis of volatile constituents. *Journal of Chromatography* **1975**, *108*.