OPTIMIZING THE CBD CRYSTALLIZATION PROCESS USING THE CRYSTAL16 MULTIPLE REACTOR SYSTEM

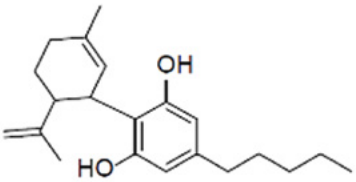
Recently, there has been a meteoric rise in cannabidiol (CBD) production from hemp as public acceptance continues to grow (Figure 1). This growth is further fuelled by favourable changes to regulations surrounding CBD-rich hemp, which has increased cultivation and production to meet the rising demand for this cannabinoid.

CBD is one of many cannabinoids produced by Cannabis sativa and has been clinically shown to reduce chronic pain and inflammation, without intoxicating effects.[1] Another major cannabinoid is the psychoactive tetrahydrocannabinol (THC) which has undesirable effects for people looking to use CBD as a medication.[2] THC is still federally illegal in the US and many other countries and needs to be removed from hemp extracts to meet regulation limits of CBD products. To ensure the purest form of CBD with as little as possible any THC contamination, recrystallization is the preferred industry standard.

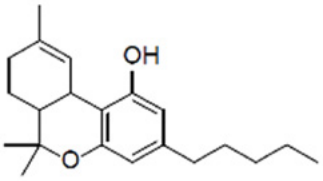
Crystallization is generally the most used industrial method for purification of organic molecules, due to its cost efficiency and

Figure 1. Chemical structure of two principal cannabinoids CBD and THC

CBD



THC



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relatively high scalability compared to other methods such as chromatography. However, the scientific literature is relatively scarce in studies detailing the CBD recrystallization process and the relevant parameters. This in turn can lead to a lack of process control, inconsistent yields, purities, and particle sizes of the CBD crystals, affecting downstream processing. The first step in understanding and designing a crystallization process for any molecule, is understanding its temperature dependent solubility and metastable zone in several solvents.

Solubility is usually represented as an amount dissolved per volume of a specific solvent at a desired temperature and pressure. This value can be measured in several ways, but perhaps the most facile one is by measuring polythermal solubility. By varying the temperature and plotting the clear points (the temperature at which all material is dissolved) and the cloud point temperatures (the point at which crystals are present) over a range of concentrations, two important curves are plotted: the metastable zone boundary in which crystals will spontaneously nucleate and form, and the solubility curve where all material is dissolved. The zone between these curves is known as the metastable zone width (MSZW).

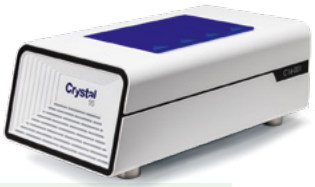
Figure 2. Pure crystalline CBD grown from a n-heptane solution

In this work we describe the solubility and MSZW behaviour of CBD in three common alkane solvents, pentane, hexane and heptane, we also describe the influence of THC contamination on the MSZW of the CBD in heptane in order to highlight the influence of impurities on this crystallization process. All experiments presented herein were performed on the ***Crystal16*** device.

Crystal16

The ***Crystal16*** is a user-friendly benchtop multi-reactor instrument to perform high throughput crystallization experiments while requiring very small amounts of material. The ***Crystal16*** can run 16 simultaneous crystallization experiments with 4 independently heated and cooled block reactors. The system has integrated transmissivity sensors enabling real-time monitoring of turbidity for each reactor/sample independently. This technology includes user-friendly software that can automatically produce clear point (dissolution) and cloud point (crystallization) temperatures, enabling rapid generation of solubility data and the metastable zone width (MSZW) with minimal amount of material at an early stage of process development for CBD crystallization and isolation.

Figure 3. Crystal16 instrument equipped with 16 parallel wells, independent heating/cooling blocks and transmissivity sensors



Specifications Crystal16	
Reactors	16
Temperature range (°C)	-20(-25) to 150 (°C)
Heating/Cooling rate (°C/min)	0-20
Stirring modes	Overhead or stir bar
Analytics	Turbidity

Results & Discussion

Influence of solvent choice

Low molecular weight hydrocarbon solvents are commonly chosen for the crystallization of CBD obtained from distillates. Butane is a popular extraction and crystallizing solvent; however, due to the major safety concerns of fire and human exposure to the solvent,[3] longer chain hydrocarbons such as n-pentane, n-hexane, and n-heptane are being increasingly adopted.

Figure 4. CBD solubility in n-pentane showing supersaturated, MSZW, and undersaturated zones

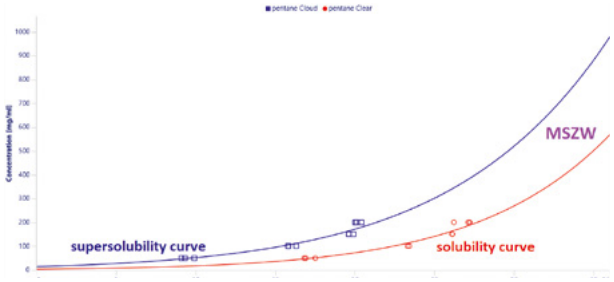
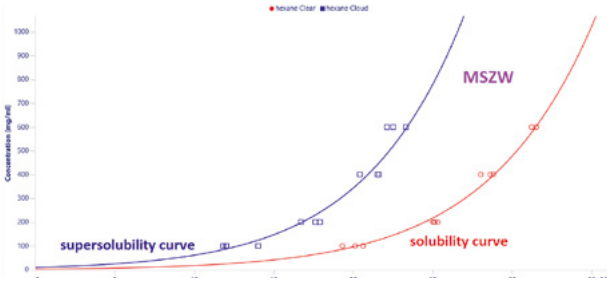


Figure 5. CBD solubility in n-hexane showing supersaturated, MSZW, and undersaturated zones



n-Pentane is currently used due its high vapor pressure and low boiling point (36.1°C), which results in easy solvent removal after the CBD crystallization. Nevertheless, pentane has a narrow MSZW which makes nucleation by seeding more difficult. The maximum concentration of dissolving CBD in n-pentane is ~550 mg/ml as the maximum temperature is the boiling point of the solvent at 36.1°C (Figure 4).

The MSZW for n-hexane is significantly larger than n-pentane allowing for more control over seed nucleating protocols during the cooling process (Figure 5). While n-hexane has a larger CBD loading capacity with a larger temperature range for dissolution, its neurotoxic properties, and potential impact on the environment, discourages its use as an appropriate CBD crystallization solvent.[4,5] The solubility profile between 5°C - 20°C of CBD in n-hexane is comparable to n-pentane, but above >20°C, the solubility dramatically increases to a maximum of ~12.370 g/ml at n-hexane's boiling point.

CBD has moderate solubility in n-heptane, compared to n-pentane and n-hexane between 5°C – 35°C. n-Heptane has the highest boiling point of the three hydrocarbons at 98.4°C and can dissolve a large volume of CBD (~88 g/mL) at this temperature (Figure 6). However, decomposition of organic compounds such as CBD can occur when exposed to >80°C and oxygen in the atmosphere, thus reducing purity and yield. Therefore, in practice, ~50°C is recommended when using n-heptane as the CBD crystallizing solvent. The MSZW of CBD in n-heptane is comparable to the n-hexane MSZW with ~12°C difference between the metastable (cloud points) and solubility curve (clear points), enabling higher control over the nucleation and crystal growth size when using cooling protocols when inducing crystallization. Currently n-heptane is not shown to be damaging to human health or the environment, and its reduced vapor pressure, and low solubility <25° C, positions it as a better solvent choice for CBD crystallization when using hydrocarbon solvents.

CBD extracted from Cannabis sativa is impure, and crystallization is often employed to produce CBD isolates from extract distillates with THC as the unwanted major constituent. To improve the purity of CBD distillate, which is typically ~80% pure to 99+% isolate, the unwanted cannabinoids are removed during the crystallization process in a hydrocarbon solvent. However, the presence of such cannabinoids in the CBD distillate can influence the crystallization parameters such as dissolution and nucleation temperatures, as well as isolated crystal yield and impurity content. We performed a series of CBD solution crystallization experiments while maintaining the CBD concentration at ~400 mg/mL in heptane, while the relative THC wt% was increased. The CBD metastable crystallization temperature decreases from 27°C for pure CBD to 15.5°C as the THC increases from 1 wt% to 16 wt%. The solubility curve was also found to decrease in temperature with an increase in THC wt%., To improve the isolated crystallization yield the solution temperature would need to reach colder temperature profiles the effect THC in CBD distillate has on crystallizing solutions. These results suggest that there is no 'one-size-fits-all' recipe when it comes to purifying CBD distillate by crystallization. To fully optimize a CBD crystallizing solution for purity and yield, each batch would need to be addressed individually as the cannabinoid content could vary between each CBD distillate run.

Experimental

Clear and cloud point temperature parameters for CBD crystallization were collected using the ***Crystal16***. CBD isolate was obtained from a Canadian licensed producer, placed in standard HPLC vials, then topped off with 1 ml solvent. The solvents studied were n-pentane, n-hexane, and n-heptane, respectively. The concentration profile for the crystallization experiments ranged from 100 mg/ml to 600 mg/ml. The CBD solutions were homogenized by heating to 40°C with magnetic stirring at 1200 rpm for five minutes. Following the homogenizing period, the CBD crystallizing solutions were cooled to 0°C at a rate of 0.5°C/minute. The crystallizing solutions were cycled through three heating and cooling ramps for reproducibility and to eliminate potential errors associated with spontaneous nucleation. In situ transmissivity measurements were used to determine the clear and cloud point temperatures for each CBD crystallization solutions. Isolated crystal yield was determined from pure CBD crystallizing solutions (100 mg/ml) at three temperatures, 22°C,

Figure 6. CBD solubility in n-heptane showing supersaturated, MSZW, and undersaturated zones

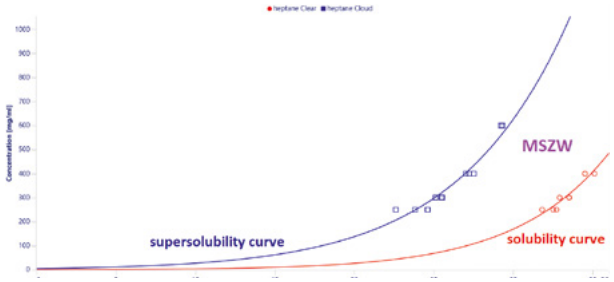


Figure 7. Influence of THC wt % on CBD crystallization parameters in n-heptane solution. CBD concentration is maintained at ~400 mg/ml

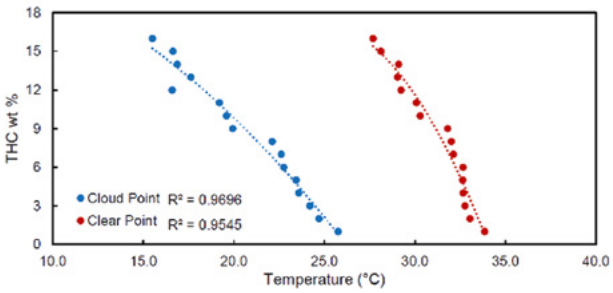


Table 1. Overview of the results

Solvent	Pros	Cons	Solubility eq.	MSZ limit eq.
Pentane	• High volatility (easy solvent removal)	• High volatility (increased risk) • Narrow MSZ • Small available temperature range	exp(43,937 – 11616,3694 / (T + 273)) (R^2 = 0,9451)	exp(38,7256 – 9838,0326 / (T + 273)) (R^2 = 0,9313)
Hexane	• Large available temperature range • Wide MSZW • High solubility	• Toxicity • Environmental concerns	exp(53,1356 – 14230,0695 / (T + 273)) (R^2 = 0.9872)	exp(54,6916 – 14312,9899 / (T + 273)) (R^2 = 0.9424)
Heptane	• Large available temperature range • Wide MSZW	• Moderate solubility (compared to n-hexane at working temperatures)	exp(59,2855 – 16408,175 / (T + 273)) (R^2 =0,9516)	exp(50,8474 – 13454,7931 / (T + 273)) (R^2 = 0,9612)

4°C, and -20°C respectively. CBD crystals were grown overnight from heptane solutions and carefully harvested and dried with the mass recorded upon reaching a constant weight. Following pure CBD crystallizing clear and cloud point temperature data acquisition for the three solvent systems, the n-heptane crystallizing solutions were contaminated with increasing THC (1 wt% – 16 wt%) while maintaining the CBD concentration at ~400 mg/ml. This was done to replicate various Cannabis sativa distillate compositions obtained prior to inducing CBD crystallization.

The work of Dr. Duane Hean, Amanda Assen and Dr. Markus Roggen shows that THC increases the solubility of CBD (and reducing crystallization temperatures) in n-heptane which needs to be considered in order to optimize cost, purity, and yield during the CBD crystallization process.

The variance from batch to batch makes the ***Crystal16*** an indispensable tool for a rapid, on-site and low-cost measurement and optimization of crystallization parameters for CBD.

Conclusions

All three solvents commonly used for CBD crystallization show significant differences in the solubility and MSZ curves. These, together with other considerations such as safety, toxicity and robustness of the crystallization process, imply the preferred solvent for CBD recrystallization is n-heptane. While it has lower maximum solubility for CBD compared to n-hexane, it has none of the health and environmental dangers associated, and, although it is less volatile than pentane, this translates to a better safety profile overall and a larger temperature working range. An overview comparison between the benefits and drawbacks of all three solvents, together with the solubility and MSZ limit equations for CBD is presented in Table 1.

Acknowledgements

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References

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