SUPERCRITICAL FLUID EXTRACTION OF CANNABIS: EXPERIMENTS AND MODELING OF THE PROCESS DESIGN

H. Perrotin-Brunel[1*], M.J.E. van Roosmalen[2], J. van Spronsen[1], R. Verpoorte[3], C.J. Peters[4] and G.J. Witkamp[1]

[1] *Process & Energy Laboratory, Faculty of Mechanical, Maritime and Materials Engineering, Delft University of Technology - Leeghwaterstraat 44 – NL-2628 CA Delft*
[2]*FeyeCon D&I B.V. – Rijnkade 17A – NL-1382 GS Weesp*
[3]*Division of Pharmacognosy – Section Metabolomics, Institute of Biology Leiden, Leiden University – P.O. Box 9502 - NL -2300 RA Leiden*
[4]*The Petroleum Institute, P.O. Box 2533, Abu Dhabi, United Arab Emirates*

h.perrotin-brunel@tudelft.nl

Abstract

Cannabis is one of the oldest medicinal plants known. At present, there is significant interest in cannabis and its medicinal uses. Cannabis contains more than 400 different ingredients, including 66 cannabinoids. The poor availability of the various cannabinoids as pure compounds is an obstacle for the development of cannabinoid based drugs and for pharmacological and toxicological studies.
Supercritical CO_2 is an excellent medium for the isolation and separation of cannabinoids from cannabis. However, to be able to design such processes, data concerning the solubility of the cannabinoids in supercritical CO_2 are required.
The solubility data for the 4 main cannabinoids - i.e. tetrahydrocannabinol, canabigerol, cannabidiol and cannabinol - in CO_2 were determined in the pressure range from 12 to 20 MPa and temperature range 315 – 334 K. Furthermore, the results were modelled using the Peng Robinson Equation of State.
Based on these data, the pressure and temperature ranges to selectively extract each cannabinoid were determined. These experimental conditions were applied to perform supercritical fluid extraction of cannabis.

Acknowledgement

Authors thank the scientific foundation STW for their financial support (project N^o LFA 7426).

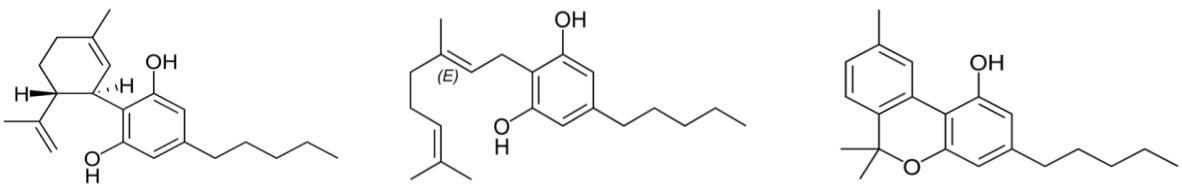
1. Introduction

At present, there is a growing interest in natural medicinal compounds. Cannabis is one of the oldest medicinal plants known [1]. Recently, the medicinal use of cannabis has been legalized in several countries. The major compound from cannabis, Δ9-THC ((-)-Δ9-tetrahydrocannabinol), has been registered for medical application in several countries and cannabis preparations are being developed as medicines. Δ9-THC is also often used as golden standard for pharmacological studies. The availability of the various cannabinoids as pure compounds is of great importance for these studies and the development of new medicines. Among various cannabinoids are cannabidiol (CBD), known to reduce neuropathic pain [2], cannabigerol (CBG) which may lower blood pressure and cannabinol (CBN) which is a decomposition product of Δ9-THC.
Cannabinoids can be extracted directly from cannabis by organic solvents (e.g. hydrocarbons and alcohols) with a yield exceeding 90% [3]. However, these solvents are flammable and many of them are toxic. Supercritical Fluid Extraction (SFE) with carbon dioxide (CO_2) is an alternative promising technique. This green solvent is widely used to extract natural components, including pharmaceutical molecules [4-8].
The application of SFE to extract cannabinoids from cannabis requires solubility data, which are currently lacking, except for Δ9-THC [9]. In this publication, the solubility measurements of CBD, CBG and CBN are presented and compared. Based on these data, the pressure and temperature ranges to selectively extract each cannabinoid were determined. These experimental conditions were applied to perform supercritical fluid extraction of cannabis.

2. Experimental

2.1. Chemicals

CO_2 was supplied by Hoek Loos (quality 2.7). CBD with a purity higher than 99% was purchased from THC Pharm (Germany). CBG and CBN, with a purity of 99.3 % and 99.5% respectively, were provided by Echo Pharmaceuticals B.V. The molecular structures of CBD, CBG and CBN are shown in Figure 1. Methanol and tetrahydofluran of HPLC reagent grade were purchased from J.T. Bakker. Cannabis plant material (female flower-tops) was medical grade and obtained from Bedrocan B.V. (Veendam, the Netherlands). The batch number was 01-57-260307. These materials were used without further purification.



CBD CBG CBN

Figure 1: Molecular structure of CBD, CBG and CBN

2.2. Apparatus and method

For the solubility experiments, a quasi-flow apparatus is used, which is described in detail elsewhere [9]. At the start of an experiment, a measured amount of compound was put into a sample cylinder and the system was closed.
Then, the oven was set at the desired temperature. After the preset temperature had been reached, the system was filled with CO_2 until the desired pressure was reached. When the conditions were stable, the CO_2 circulation over the sample vessel started. A sample for HPLC analysis was taken after 4 hours and successively every 30 minutes. When the concentration difference measured was less than $0.09x10^{-4}$ between two subsequent analyses, with a pressure and temperature differences less than 0.05 MPa and 0.2 K respectively, it was assumed that equilibrium was reached, and the concentration measured was recorded as the solubility.

2.3. High-Performance Liquid Chromatography

The HPLC profiles were acquired on a Chromapack HPLC system consisting of an Isos pump, an injection valve and a UV-VIS detector (model 340 – Varian). The system is controlled by Galaxie Chromatography software. The profiles were recorded at 228 nm.
The analytical column was a Vydac (Hesperia, CA) C_{18}, type 218MS54 (4.6 * 250 mm^2, 5 μm). The mobile phase consisted of a mixture of methanol, distilled water and tetrahydrofluran in the proportions v/v/v = 10/4/1. The flow rate was 1.5 $mL.min^{-1}$ and the total running time was 14 minutes.

2.4. Supercritical Fluid Extraction

Figure 2 shows a schematic overview of the pilot plant used for the cannabinoids extraction with supercritical CO_2 [10, 11]. During a run, the cooling and heating system are switched on first, and set to the desired temperature. Next, the extraction vessel is opened and filled with approx. 45 g decarboxylated cannabis with a Δ9-THC content of around 13%. After closing the vessel, CO_2 is continuously pumped (flow rate is 6 $kg.h^{-1}$) from the storage vessel into the extraction vessel, which is kept at the required temperature by using a heating jacket. At the moment that the desired pressure is reached, the pressure transducer starts controlling the CO_2 flow into the separator. Via a condenser, the CO_2 is recycled to the storage vessel. During extraction, samples can be taken from the separator. After an extraction run, the extract is weighed and analyzed using the previously described HPLC method. The remaining residue was also weighed to check the mass balance.

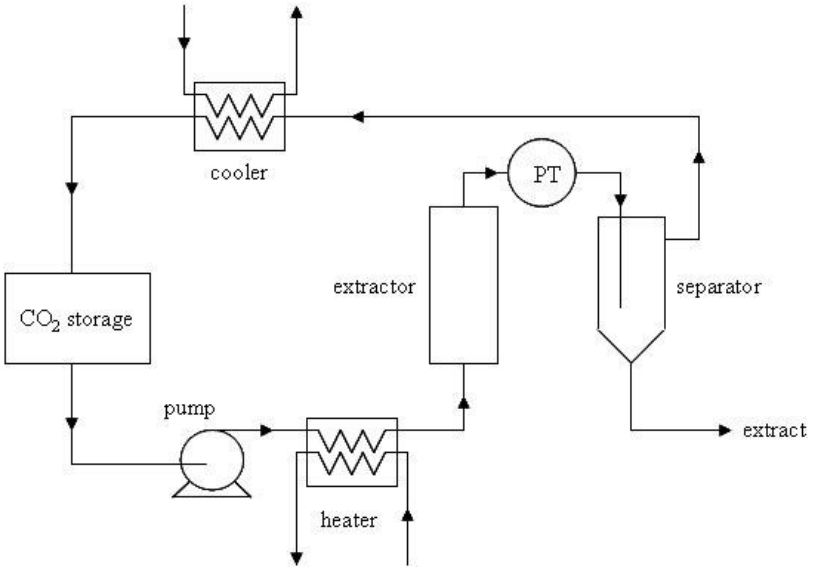


Figure 2: Schematic overview of the experimental set-up for the supercritical extraction of cannabis

3. Results and discussion

3.1 Solubility data

The experimental solubility data of CBD, CBG and CBN in CO_2 are shown in Table 1. The maximum standard deviation was 0.0015 x 10^{-4}. Figure 2 represents the solubility data of the 4 considered cannabinoids in this study, i.e. Δ9-THC, CBD, CBG and CBN, at 327 K.

Table 1: Molar solubility (*x*) of CBD, CBG and CBN at different temperatures and pressures

Compound	*T = 314 K*		*T = 326 K*		*T = 334 K*	
	P (MPa)	**10^4 *x***	***P (MPa)***	**10^4 *x***	***P (MPa)***	**10^4 *x***
CBD	11.3	1.00	11.8	0.94	11.4	0.88
314 g.mol^{-1}	14.3	1.66	15.5	2.37	16.4	1.79
	17.3	1.85	19.4	2.69	18.8	1.84
CBG	13.4	1.17	15.3	1.23	15.4	1.37
316 g.mol^{-1}	18.4	1.23	17.8	1.32	18.5	1.82
	20.6	1.28	20.1	1.49	19.1	1.91
CBN	14.5	1.27	14.0	3.74	14.6	2.10
310 g.mol^{-1}	17.7	1.77	16.6	4.08	17.4	2.17
	19.1	2.08	17.8	4.16	19.9	2.17

For all these cannabinoids, the solubility increases with a pressure increase. Δ9-THC has the lowest solubility. The solubility increases in the following order: Δ9-THC < CBG < CBD < CBN. CBN has the most stable molecular structure and less polar OH groups available. Therefore, this compound is less polar than the other cannabinoids, which explains the higher solubility in CO_2. CBG has the highest molecular weight,

therefore it seems to be logical that its solubility is lower than the solubility of CBD and CBN.

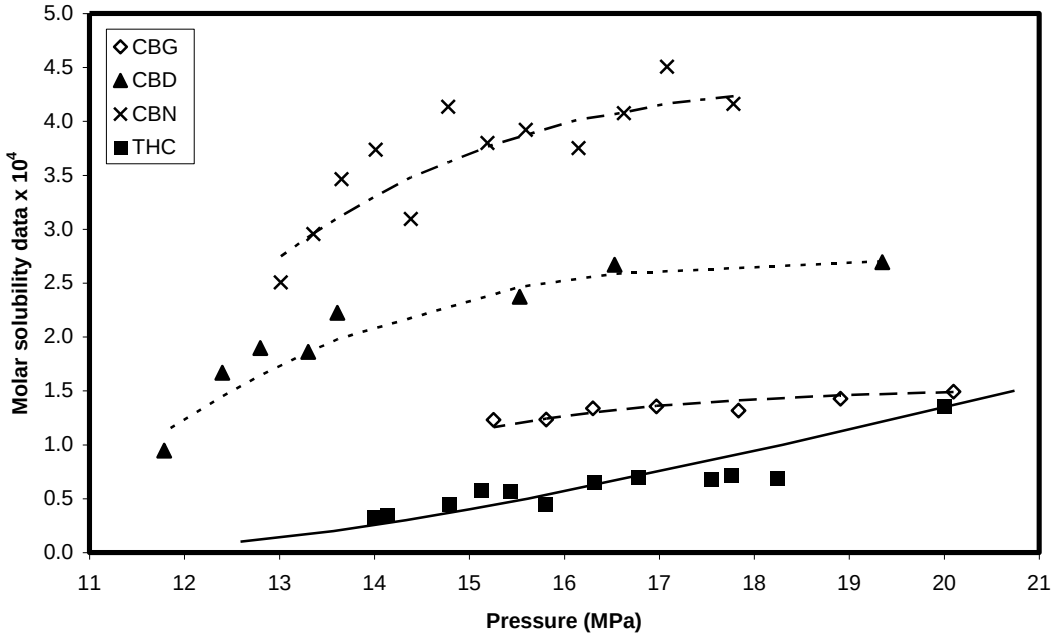


Figure 3: Solubility data of THC, CBD, CBN and CBG at 327 K

As the solubility of the various cannabinoids is different at the same temperature and pressure, it should be possible to get extracts with different cannabinoid amounts at different conditions.

3.2 Supercritical Fluid Extraction – preliminary results

The preliminary results of the Δ9-THC extraction using supercritical CO_2 are presented in Table 2. In all cases, an extraction efficiency for Δ9-THC of over 50% was achieved. The highest Δ9-THC content of the extract is found at 230 bar, which is consistent with the solubility results found earlier [9].

Table 2: The effect of pressure (*p*), temperature (*T*) and time (*t*) on the extraction efficiency (η) of Δ9-THC.

p / bar	*T / °C*	*t / min*	η / %
150	40	127	73.9
200	40	180	63.1
230	40	180	91.4
150	50	195	71.4
200	50	180	52.5
230	50	180	67.8

The Δ9-THC content of the product is around 50%, which is too low for direct use in a pharmaceutical product where a Δ9-THC content of > 95% is required.

As the other cannabinoids were present in low quantity compared to Δ9-THC, it was difficult to quantify them in the extract. Further investigations have to be done in order to improve the separation process and obtain a higher purity.

4. Conclusion

In this work, the solubility of CBD, CBG and CBN have been determined experimentally. From these data, pressure and temperature could be chosen to perform SFE in order to extract cannabinoids. As the cannabis plant was containing a lot of Δ9-THC compared to the other cannabinoids, it was not possible to obtain a selective process to isolate one particular cannabinoid. The extracts were containing in average 50% of Δ9-THC. A further purification step is needed to obtain an extract pure enough to be used in the pharmaceutical industry.

References

[1] A. W. Zuardi, Revista Brasileira De Psiquiatria 28 (2006) 153-157.
[2] C. R. McAllister SD, Horowitz MP, Garcia A, Desprez PY, Mol. Cancer Ther 6 (2007) 2921.
[3] A. Hazekamp, R. Simons, A. Peltenburg-Looman, M. Sengers, R. van Zweden, R. Verpoorte, Journal of Liquid Chromatography & Related Technologies 27 (2004) 2421-2439.
[4] R. A. D. Mendes R.L., Pereira A.P., Cardoso M.T., Palavra A.F., Coelho J.P., Chemical Engineering Journal 105 (2005) 147-152.
[5] E. Reverchon, Journal of Supercritical Fluids 10 (1997) 1-37.
[6] E. Reverchon, I. De Marco, Journal of Supercritical Fluids 38 (2006) 146-166.
[7] H. Sovová, M. Sajfrtová, M. Bártlová, L. Opletal, The Journal of Supercritical Fluids 30 (2004) 213-224.
[8] I. Zizovic, M. Stamenic, A. Orlovic, D. Skala, The Journal of Supercritical Fluids 39 (2007) 338-346.
[9] H. Perrotin-Brunel, P. C. Perez, M. J. E. van Roosmalen, J. van Spronsen, G.-J. Witkamp, C. J. Peters, The Journal of Supercritical Fluids 52, (2010) 6-10.
[10] C.Kersch, M.van. der. Kraan, G.F.Woerlee, G.J.Witkamp, J.Chem. Technol. Biotechnol. 77 (2002) 256-259.
[11] C.Kersch, M. J. E. van Roosmalen, G.F. Woerlee, G.J. Witkamp, Ind. Eng. Chem. Res 39 (2000) 4670 - 4672.