3704 *J. Chem. Eng. Data* **2010,** *55,* 3704–3707

Solubility of Cannabinol in Supercritical Carbon Dioxide

Helene Perrotin-Brunel,*,† Maaike J. E. van Roosmalen,‡ Maaike C. Kroon,† Jaap van Spronsen,†
Geert-Jan Witkamp,† and Cor J. Peters†,§

Laboratory for Process Equipment Section, Process and Energy Department, Faculty of Mechanical, Maritime and Materials Engineering, Delft University of Technology, Leeghwaterstraat 44, 2628 CA Delft, The Netherlands, FeyeCon D&I B.V., Rijnkade 17a, 1382 GS Weesp, The Netherlands, and The Petroleum Institute, P.O. Box 2533, Abu Dhabi, United Arab Emirates

---

Cannabinol (CBN) is a decomposition product of the cannabinoid (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), the main active compound of cannabis. The solubility of CBN in supercritical carbon dioxide was determined at (314, 327, and 334) K and in the pressure range from (13.0 to 20.2) MPa by using an analytical method with a quasi-flow apparatus. The molar solubility of CBN ranged from $1.26 \cdot 10^{-4}$ to $4.16 \cdot 10^{-4}$. CBN showed different behavior compared to Δ^9-THC in terms of molar solubility. The data were correlated using the Peng−Robinson equation of state in combination with quadratic mixing rules. Deviations between calculated results and the experimental data ranged from (4.14 to 4.46) % absolute average relative deviation (AARD).

---

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 biologically active compound from cannabis, the cannabinoid (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), has been registered for medical applications, and cannabis preparations are being developed as medicines. Δ^9-THC eases pain and is neuroprotective; it has approximately equal affinity for the CB1 and CB2 receptors. Its effects are perceived to be mostly cerebral.[2] However, Δ^9-THC is not the only biologically active compound in cannabis. In total, cannabis contains more than 400 different ingredients, including 66 cannabinoids that can show biological activity.[2]

One of these cannabinoids is cannabinol (CBN). CBN is only mildly psychoactive and is perceived to be sedative or stupefying. It is the primary product of Δ^9-THC degradation, and its amount is limited in a fresh plant. CBN content increases as Δ^9-THC degrades in storage under exposure to light and air. This chemical reaction is a dehydrogenation reaction and is represented in Figure 1. The cyclohexene ring present in Δ^9-THC is dehydrogenated to become an aromatic benzoic ring.[3]

To obtain pure cannabinoids, they can be extracted directly from cannabis by organic solvents (e.g., hydrocarbons such as hexane and alcohols) with a yield exceeding 90 %.[4] However, these solvents are flammable, and many of them are toxic. Supercritical fluid extraction (SFE) with carbon dioxide (CO_2) is a promising alternative technique. There are no flammability or toxicity issues; solvent removal is simple and efficient, and the extract quality can be well-controlled. This green solvent has widely been used for the extraction of natural compounds, including pharmaceutical molecules, from plant material.[5−11]

To extract cannabinoids from cannabis with the use of SFE, it is crucial to have solubility data. Such data are however currently lacking. So far, only the solubility of Δ^9-THC has been reported.[12]

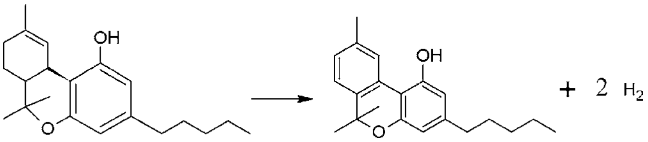

Figure 1. Dehydrogenation of Δ^9-THC into CBN.

To reduce the lack of solubility data of cannabinoids, this work presents the determination of the solubility of CBN in supercritical CO_2. In addition, the experimental data are correlated using the Peng−Robinson equation of state (PR-EoS). Finally, the solubilities of CBN and Δ^9-THC in supercritical CO_2 are compared, and their differences are explained in terms of structure, molecular weight, and polarity.

2. Experimental Section

The solubility of CBN in supercritical CO_2 was measured at (314, 327, and 334) K and pressures between (13.0 and 20.2) MPa, by using an analytical method with a quasi-flow apparatus. Details of this solubility cell and the equipment for analyses can be found elsewhere.[12]

The solubility cell was loaded by transferring a liquid mixture of CBN and methanol into the sample cylinder, after which the methanol was evaporated with a vacuum pump (RNF Lab) for 1 h at ambient temperature to ensure complete evaporation of the solvent. Subsequently, the pump was disconnected, and the system was closed. CO_2 at the desired temperature was added to the solubility cell filled with CBN until the desired pressure was reached and the CO_2 circulation over the sample vessel was started. The temperature measurements have an uncertainty of 0.2 K due to the temperature fluctuations in the oven and the error in the reading of the thermometer. The uncertainty of the pressure measurements is 0.05 MPa.

A sample for high-performance liquid chromatography (HPLC) analysis was taken after 4 h and successively every 30 min. When the concentration difference measured was less than $0.09 \cdot 10^{-4}$ mol·mol^{-1} between two subsequent analyses, it was

---

* Corresponding author. Phone: +31 152785561. Fax: +31 152786975. E-mail: h.perrotin-brunel@tudelft.nl.
† Delft University of Technology.
‡ FeyeCon D&I B.V.
§ The Petroleum Institute.

10.1021/je100245n

Table 1. Molar Solubility Data (y) of CBN at Different Temperatures (T) and Pressures (P)

T/K = 314		T/K = 327		T/K = 334	
P/MPa	$10^4\ y$	P/MPa	$10^4\ y$	P/MPa	$10^4\ y$
13.8	1.26 ± 0.13	13.0	2.51 ± 0.25	13.0	1.38 ± 0.14
14.3	1.27 ± 0.13	13.4	2.95 ± 0.30	13.3	1.33 ± 0.13
14.5	1.27 ± 0.13	13.7	3.46 ± 0.35	13.6	1.65 ± 0.16
15.3	1.47 ± 0.15	14.0	3.74 ± 0.37	14.6	2.10 ± 0.21
15.5	1.58 ± 0.16	14.4	3.09 ± 0.31	14.9	1.99 ± 0.20
16.3	1.79 ± 0.18	14.8	4.14 ± 0.41	15.6	2.35 ± 0.23
17.7	1.77 ± 0.18	15.2	3.80 ± 0.38	16.5	2.27 ± 0.23
18.2	2.02 ± 0.20	15.6	3.92 ± 0.39	17.1	2.48 ± 0.25
19.1	2.08 ± 0.21	16.2	3.75 ± 0.38	17.4	2.17 ± 0.22
20.2	2.33 ± 0.23	16.6	4.08 ± 0.41	18.2	2.20 ± 0.22
		17.1	4.51 ± 0.45	18.5	2.36 ± 0.24
		17.8	4.16 ± 0.42	19.9	2.17 ± 0.22

assumed that equilibrium was reached, and the concentration measured was recorded as the solubility.

The HPLC 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 tetrahydrofuran in the proportions 0.62:0.31:0.07 (mass fractions). The flow rate was 1.5 mL·min^{-1}, and the total running time was 14 min. Because the peak areas of the components calculated from the chromatograms are linearly related to their amounts by the Lambert−Beer law, it was possible to determine their concentration using a calibration line. This line was realized by using five standard samples with different concentrations in the range (0 to 5) mg·mL^{-1}. Each standard sample was injected at least three times, and an average was taken to perform the linear regression. The linear regression coefficient of the calibration curve was equal to 0.997.

The CO_2 used for the measurements was supplied by Hoek Loos (quality 2.7). CBN with a purity of 0.995 mass fraction was provided by Echo Pharmaceuticals B.V. Methanol and tetrahydrofuran (HPLC reagent grade) were purchased from J. T. Bakker. These materials were used without further purification.

3. Results and Discussion

The solubility of CBN in supercritical CO_2 was measured at (314, 327, and 334) K and in the pressure range from (13.0 to 20.2) MPa. The results in term of molar fractions (y) are summarized in Table 1 and graphically shown in Figure 2. Each point is an average of at least two measurements. The maximum standard deviation was 0.0002·10^{-4}, as represented by the error bars in Figure 2.

As shown for the isotherms in Figure 2, the solubility of CBN in supercritical CO_2 increases with an increase in pressure. Interestingly, the highest solubility is observed at the medium temperature (327 K), while it was expected that the solubility would increase with increasing temperature, just as was observed for Δ^9-THC.[12] Although uncommon, this phase behavior is theoretically possible and has been observed before, for example, in the naphthalene + supercritical ethylene system.[13]

Also, contrary to Δ^9-THC[12], no crossover region was observed in the measured pressure range. However, this behavior is likely to occur at pressures lower than the lowest pressure in the measurements (13.0 MPa), because it is expected that the solubility curves intercept around 10 MPa (extrapolation of Figure 2).

The experimental data were correlated with the PR-EoS:[14,15]

$$P = \frac{RT}{V - b} - \frac{a}{V^2 + 2bV - b^2} \tag{1}$$

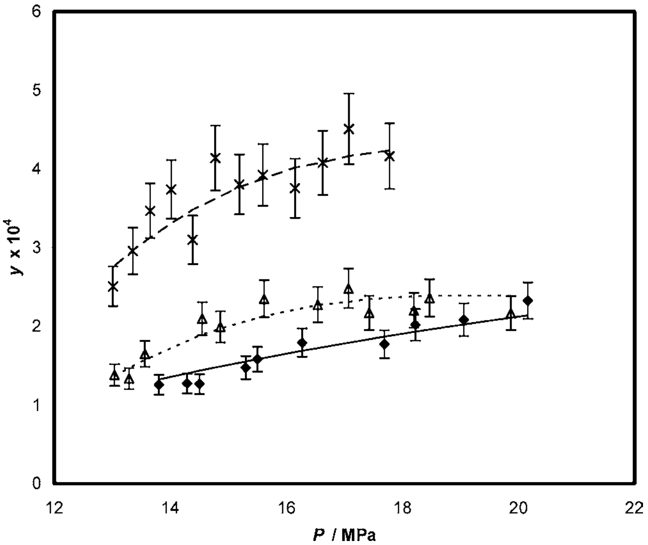

Figure 2. CBN molar solubility (y) in supercritical CO_2. Experimental: ◆, 315 K; ×, 327 K; △, 334 K. −, correlated with the PR-EoS.

Table 2. Critical Temperatures (T_c), Critical Pressures (P_c), and Acentric Factors (ω) Used in the PR-EoS

substance	T_c/K	P_c/MPa	ω
CO_2	304.4	7.38	0.225
CBN	920	1.65	0.431
Δ^9-THC	988	1.95	0.882

Table 3. Binary Parameters (k_{ij}) Including Absolute Average Relative Deviation (AARD) for the Binary CBN + CO_2 System at Different Temperatures (T)

T/K	k_{ij}	AARD/%
314	0.113	4.21
326	0.173	4.46
334	0.212	4.14

where P is the pressure, T is the temperature, V is the volume, R is the gas constant, and a and b are parameters calculated from the quadratic mixing rule. The attractive term is given by:

$$a = \sum_i \sum_j y_i y_j (a_i a_j)^{0.5}(1 - k_{ij}) \tag{2}$$

where k_{ij} is the binary interaction parameter to be optimized and

$$a_i = 0.45724 \frac{R^2 T_{ci}^2}{P_{ci}} \alpha_i \tag{3}$$

where

$$\alpha_i = [1 + m(\omega_i)(1 - T_{ri}^{1/2})]^2 \tag{4}$$

where

$$m(\omega_i) = 0.37464 + 1.54226\omega_i - 0.26992\omega_i^2 \tag{5}$$

The covolume parameter is given by:

$$b = \sum_i y_i b_i \tag{6}$$

where

$$b_i = 0.07780 \frac{RT_{ci}}{P_{ci}} \tag{7}$$

To use these equations, the critical properties (T_c, P_c) and acentric factor (ω_i) of the components are required. However, critical properties of CBN are not available in literature.

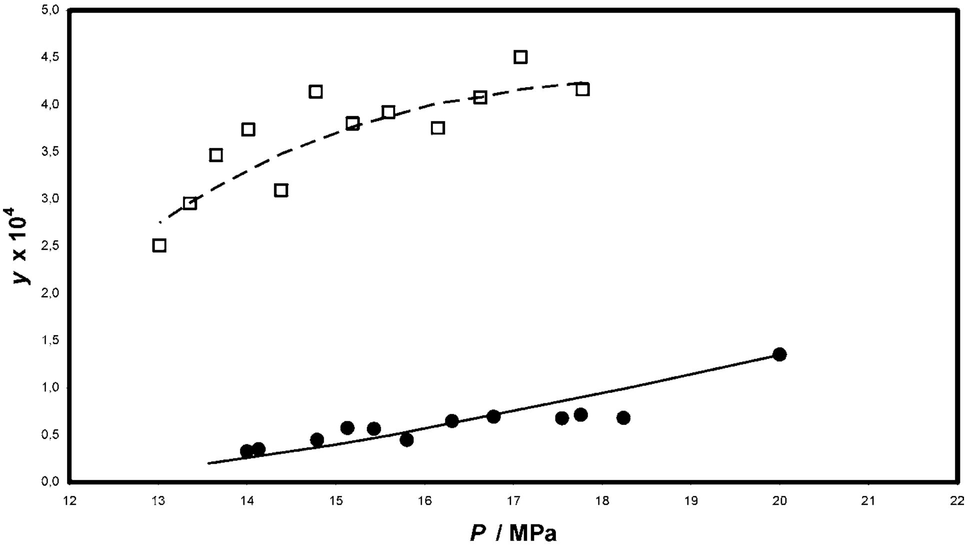

Figure 3. THC, ●, and CBN, □, molar solubilities (y) in CO_2 at 327 K.

Therefore, these properties have been estimated using the Gani method,[16] and the values for CO_2 were taken from the PE database.[17] The critical properties and acentric factors of CBN and CO_2 are shown in Table 2. The critical properties of Δ^9-THC[12] are also presented for comparison. As can be seen in Table 2, the values for the critical pressure and temperature of CBN are in the same order of magnitude as Δ^9-THC. However, Δ^9-THC has a higher acentric factor because of the absence of the aromatic ring that is present in CBN.[14]

The binary interaction parameter k_{ij} was calculated from the experimental pressure at each point by minimizing the relative difference between experimental and calculated pressure.[14] This minimization can be expressed by the absolute average relative deviation (AARD (%)), as described by the following equation:

$$\text{AARD}\ (\%) = \frac{100}{n}\sum_{1}^{n}\frac{|p_i^{\text{exp}} - p_i^{\text{calc}}|}{p_i^{\text{exp}}} \qquad (8)$$

Here, n is the number of data experiments at each temperature, and P_i^{exp} is the experimental pressure for the experiment i, whereas P_i^{calc} is the estimated value. The AARD values at different temperatures are presented in Table 3. Their values, around 4 %, show that the data are well correlated by the PR-EoS.

Table 3 also presents the binary parameter k_{ij}, at the different temperatures. This parameter increases linearly with a rise in temperature. The regression coefficient had a value of 1.000. This demonstrates the consistency of the experimental results.

In Figure 3, the solubility of CBN in supercritical CO_2 at 327 K is compared to the solubility data of Δ^9-THC in supercritical CO_2 at the same temperature from literature.[12] This figure shows that the solubility of Δ^9-THC is lower than the solubility of CBN. This behavior is observed at any measured temperature. This can be explained by the lower polarity of CBN compared to Δ^9-THC, which increases the affinity for the nonpolar supercritical CO_2. Moreover, the lower molar mass of CBN compared to Δ^9-THC also increases its solubility in supercritical CO_2, although the effect is probably small (only 4 $g \cdot mol^{-1}$ difference).

From these data it may be concluded that, if a cannabis plant (after storage) contained both CBN and Δ^9-THC, both cannabinoids could be separated from each other with supercritical CO_2 on basis of their different affinity. CBN could be extracted first at low pressure (i.e., around 13 MPa), after which the active Δ^9-THC could be extracted at higher pressures (around 20 MPa). This could be a selective process to isolate CBN separately from Δ^9-THC.

4. Conclusion

In this work, the solubility of the cannabinoid CBN in supercritical CO_2 was measured at temperatures between (314 and 334) K and a pressure range from (13.0 to 20.2) MPa. The highest solubility was observed at the highest pressures and intermediate temperature (327 K). This behavior is different from the solubility of another cannabinoid, Δ^9-THC, in CO_2, which shows the highest solubility at the highest temperature. The experimental data can be adequately represented by the PR-EoS. As expected from its structure, molecular weight, and polarity, CBN is more soluble than Δ^9-THC in supercritical CO_2 in the studied pressure and temperature ranges. Therefore, it can be concluded that supercritical CO_2 may be a good solvent to isolate CBN from Δ^9-THC by extraction.

Literature Cited

(1) Zuardi, A. W. History of cannabis as a medicine: a review. *Rev. Bras. Psiquiatr.* **2006**, *28* (2), 153–157.

(2) Russo, E. Introduction to Cannabis: from Pariah to prescription. *J. Cannabis Ther.* **2003**, *3*, 1–19.

(3) Bastola, K. P.; Hazekamp, A.; Verpoorte, R. Synthesis and spectroscopic characterization of cannabinolic acid. *Planta Med.* **2007**, *73*, 273–275.

(4) Hazekamp, A.; Simons, R.; Peltenburg-Looman, A.; Sengers, M.; van Zweden, R.; Verpoorte, R. Preparative Isolation of Cannabinoids from Cannabis sativa by Centrifugal Partition Chromatography. *J. Liq. Chromatogr. Relat. Technol.* **2004**, *27*, 2421–2439.

(5) Mendes, R. L.; Reis, A. D.; Pereira, A. P.; Cardoso, M. T.; Palavra, A. F.; Coelho, J. P. Supercritical CO_2 extraction of α-linolenic acid (GLA) from the cyanobacterium Arthrospira (Spirulina) maxima: experiments and modeling. *Chem. Eng. J.* **2005**, *105*, 147–152.

(6) Reverchon, E. Supercritical fluid extraction and fractionation of essential oils and related products. *J. Supercrit. Fluids* **1997**, *10*, 1–37.

(7) Reverchon, E.; De Marco, I. Supercritical fluid extraction and fractionation of natural matter. *J. Supercrit. Fluids* **2006**, *38*, 146–166.

(8) Sovová, H.; Sajfrtová, M.; Bártlová, M.; Opletal, L. Near-critical extraction of pigments and oleoresin from stinging nettle leaves. *J. Supercrit. Fluids* **2004**, *30*, 213–224.

(9) Zizovic, I.; Stamenic, M.; Orlovic, A.; Skala, D. Supercritical carbon dioxide extraction of essential oils from plants with secretory ducts: Mathematical modelling on the micro-scale. *J. Supercrit. Fluids* **2007**, *39*, 338–346.

(10) Coimbra, P. C.; Fernandes, D.; Gil, M. H.; Sousa, H. C. Solubility of Diflunisal in Supercritical Carbon Dioxide. *J. Chem. Eng. Data* **2008**, *53*, 1990–1995.

(11) Su, B.; Xing, H.; Yang, Y.; Ren, Q. Solubility of Oxymatrine in Supercritical Carbon Dioxide. *J. Chem. Eng. Data* **2008**, *53*, 1672–1674.

(12) Perrotin-Brunel, H.; Cabeza Perez, P.; van Roosmalen, M. J. E.; van Spronsen, J.; Witkamp, G.-J.; Peters, C. J. Solubility of [Δ]9-tetrahydrocannabinol in supercritical carbon dioxide: Experiments and modeling. *J. Supercrit. Fluids* **2010**, *52*, 6–10.

(13) Diepen, G. A. M.; Scheffer, F. E. C. The Solubility of Naphthalene in Supercritical Ethylene. II. *J. Phys. Chem.* **1953**, *57*, 575–577.

(14) Montequi, I.; Alonso, E.; Martin, A.; Cocero, M. J. Solubility of Diisopropoxititanium Bis(acetylacetonate) in Supercritical Carbon Dioxide. *J. Chem. Eng. Data* **2008**, *53*, 204–206.

(15) Peng, D. Y.; Robinson, D. B. A new two constant equation of state. *Ing. Eng. Chem. Fundam.* **1976**, *15*, 59–64.

(16) Poling, B.; O'Cornell, J. P.; Prausnitz, J. M. *The properties of gases and liquids*, 5th ed.; McGraw-Hill: New York, 2000.

(17) Pfohl, O. P. S.; Brunner, G. PE quickly makes available the newest equations of state via internet. *Ind. Eng. Chem. Res.* **2000**, *39*, 4439.

Received for review March 16, 2010. Accepted May 17, 2010. Financial support by STW (Project No. LFA 7426) is gratefully acknowledged.

JE100245N