Journal of Current Science and Technology, April - June 2025 Vol. 15 No. 2, Article 98

ISSN 2630-0656 (Online)

Cite this article: Chankana, C., Monton, C., Naksuriya, O., & Songsak, T. (2025). Optimization of solvent mixture for solubilizing cannabis extract using I-optimal design. *Journal of Current Science and Technology*, *15*(2), Article 98. https://doi.org/10.59796/jcst.V15N2.2025.98

Journal of Current Science and Technology

Journal homepage: https://jcst.rsu.ac.th

Optimization of Solvent Mixture for Solubilizing Cannabis Extract Using I-Optimal Design

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Received 3 October 2024; Revised 11 November 2024; Accepted 15 November 2024; Published online 25 March 2025

Abstract

Cannabis extract is a poorly water-soluble but alcohol-soluble substance. The development of a cannabis-based oral spray using a high ethanol concentration could cause oromucosal irritation. Finding a suitable solvent mixture to dissolve cannabis extract is challenging. This work aimed to evaluate the solubilization characteristics of cannabis extract in different solvent mixtures commonly used in oral spray formulations. A 0.1 g sample of cannabis extract, obtained through supercritical carbon dioxide fluid extraction and containing 14.09% cannabidiol and 34.19% Δ^9-tetrahydrocannabinol, was dissolved in 1 g of a solvent mixture. The mixture of three solvents (ethanol, propylene glycol, and water) was varied using an I-optimal design. Results showed that the solvent system promoted a clear solution with maximum recovery of cannabidiol and Δ^9-tetrahydrocannabinol, composed of 53% ethanol, 35% propylene glycol, and 12% water. This optimized solvent mixture was further verified, and a minor percentage error of less than 10% was achieved, indicating that the software-generated prediction was accurate and reliable. In conclusion, this work clarifies the effect of the solvent mixture on the solubilization characteristics of cannabis extract, which is valuable for the development of a cannabis-based oral spray for the treatment of several ailments.

Keywords**:** *cannabidiol; design of experiments; oral spray; Δ^9-Tetrahydrocannabinol; supercritical carbon dioxide fluid*

1. Introduction

Cannabis or marijuana (*Cannabis sativa* L. subsp. *indica*) belongs to the Cannabaceae family. It contains 750 identified phytochemicals, among which, approximately 100 compounds have been identified as cannabinoids (Radwan et al., 2015). It contains two key neutral cannabinoids: cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC). Currently, CBD and Δ^9-THC are phytocannabinoids that have been used for medicinal purposes (Madras, 2015). The primary use of cannabis is for the treatment of anxiety, appetite loss, chronic pain, depression, and insomnia (Hazekamp et al., 2013). Extensive research, including systematic reviews and meta-analyses, indicates that cannabis can effectively treat multiple diseases and provide relief from their associated symptoms, e.g., chronic pain, nausea and vomiting, epilepsy, inflammatory bowel disease, insomnia, multiple sclerosis, neuropathic pain, parkinsonism, psoriasis, spasticity, substance use disorders, Tourette syndrome, and weight loss, etc. (Aviram, & Samuelly-Leichtag, 2017; Bilbao, & Spanagel, 2022; Charoenying et al., 2023; Machado Rocha et al., 2008; Solmi et al., 2023; Martin-Sanchez et al., 2009; Nugent et al., 2017; Pamplona et al., 2018; Stockings et al., 2018a, 2018b; Ware et al.,

2010; Whiting et al., 2015). Moreover, Thai traditional medicines containing cannabis have long been used such as the Suk Sai Yas formula (Pathamaporn et al., 2022) and Ammarit-Osot formula (Wunnakup et al., 2024).

Recently, several cannabis-based products have entered on the market. For example, Sativex®, an oral spray containing 38-44 mg and 35-42 mg of two cannabis extracts, which is equivalent to 27 mg Δ^9-THC and 25 mg CBD in a 100 mL solution, has been approved for treating moderate to severe spasticity due to multiple sclerosis (GW Pharma, 2024). Epidiolex® is an oral solution containing 100 mg/mL CBD, used for treating seizures associated with Lennox-Gastaut syndrome, Dravet syndrome, or tuberous sclerosis (Jazz Pharmaceuticals, 2024). Xativa® is a sublingual wafer tablet containing 12.5 mg CBD, indicated for pain relief, nausea, and certain motor disorders (iX Biopharma, 2024; Therapeutic Goods Administration, 2021). In Thailand, cannabis oral drops with varying CBD and Δ^9-THC ratios manufactured by the Government Pharmaceutical Organization have also been launched. Additionally, other innovative cannabis products are in the research and development phase, such as transdermal patch (Sermsaksasithorn et al., 2024; Wongwad et al., 2024), transdermal nanocarriers (Chu et al., 2024), liquisolid tablet (Jaipakdee et al., 2022; Limpongsa et al., 2022), self-emulsifying drug delivery system (Monton et al., 2024b; Monton et al., 2022), etc.

Oromucosal delivery, where drugs enter the bloodstream through mouth tissues, offers numerous advantages: easy administration, high patient acceptance, avoidance of first-pass metabolism, reduced drug degradation in the stomach, and the ability to quickly remove the drug if side effects occur (Zhang et al., 2002). However, CBD and Δ^9-THC have poor water solubility. CBD has a water solubility of 12.6 µg/mL with high lipophilicity (log P of 6.3) (Grifoni et al., 2022), however, another study reported a solubility of 0.1 µg/mL (Mannila et al., 2007). In addition, CBD solubilities in ethanol and methanol are 30 and 35 mg/mL, respectively (Cayman Chemical, 2015). Δ^9-THC has a water solubility of 2.8 µg/mL, alcohol solubility of 1 part in 1 part of alcohol, and glycerin solubility of 1 part in 3 parts of glycerin (World Health Organization, 2018). An oral spray is a liquid formulation designed to be dispensed into the oral cavity, targeting the oromucosal area for absorption. It typically consists of an active ingredient, along with a solvent and cosolvent to aid in solubilization, as well as additional excipients like flavoring agents, preservatives, and stabilizers to enhance stability, taste, and shelf life. However, selecting an appropriate solvent and cosolvent system for solubilizing cannabinoids like CBD and Δ^9-THC in liquid vehicles presents challenges. High ethanol content, often used to improve solubility, can irritate the oromucosal tissues, whereas high water content can result in a poorly soluble, unstable mixture that limits the bioavailability of the active ingredients.

Mixture designs are a type of response surface design focused on the optimization process, which is essential for creating designs that meet specific criteria. In mixture experiments, components are combined in various proportions, each ranging from zero to one, with the total proportion of all components equaling one (Hamim Wigena et al., 2019). In mixture designs, the I-optimal design is oriented toward improving the precision of response predictions (Jones et al., 2021; Zhao et al., 2023). By minimizing the average variance of predictions across the experimental region, I-optimal designs place a strong emphasis on predictive accuracy. Consequently, the I-optimality criterion is often considered more suitable than the D-optimality and A-optimality criteria for constructing response surfaces (Jones, & Goos, 2012).

This work aimed to apply the mixture design, specifically the I-optimal mixture design, to identify solvent mixtures of ethanol, propylene glycol (PG), and water that would completely dissolve cannabis extract. Modern experimental design, compared to traditional methods, offers substantial benefits in terms of time, budget, and resource savings due to fewer experimental runs. It also facilitates the identification of factor interactions and the characterization of response surfaces (Gibson, 2016; Steele, 2018). Additionally, this approach allows for statistical modeling, enabling the prediction of multiple factor effects simultaneously (JMP Statistical Discovery LLC, 2022). The authors expected that this work could clarify the effect of the solvent mixture on the solubilization characteristics of cannabis extract, which is valuable for the development of a cannabis-based oral spray for the treatment of several ailments.

2. Objectives

This work aimed to apply the mixture design, specifically the I-optimal mixture design, to find solvent mixtures of ethanol, PG, and water that could completely dissolve cannabis extract.

3. Materials and Methods

3.1 Materials

CBD and Δ^9-THC standards were obtained from the Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand. Cannabis inflorescences were obtained from the Office of Narcotics Control Board, Ministry of Justice, Thailand. Ethanol (99.8%) was purchased from the Liquor Distillery Organization, Chachoengsao, Thailand. PG was purchased from P.C. Drug Center Co., Ltd., Bangkok, Thailand. Ultrapure water was produced by Direct Q 3 UV system, Merck Ltd., Bangkok, Thailand. Methanol (HPLC grade) was purchased from RCI Labscan Limited, Bangkok, Thailand.

3.2 Preparation of Cannabis Extract

Cannabis inflorescence powder was extracted using supercritical carbon dioxide fluid extraction using the optimized condition followed by the previous work. Cannabis powder (600 g) was extracted using a supercritical carbon dioxide extractor with a 5 L extraction vessel (ExtrateX, Pont-Saint-Vincent, France). The extraction was conducted at a pressure of 18 MPa and a vessel temperature of 40°C, without ethanol as a modifier. Separator I and II temperatures were set at 65°C and 45°C, respectively, while the temperature of separator III was uncontrolled. The extract was collected after 1 h of extraction. The extract underwent a winterization process which involved dissolving it in ethanol, freezing, and filtration. The ethanol was then removed using a rotary evaporator, resulting in a clear amber extract (Monton et al., 2022). This extract was proven to contain no heavy metals, pesticides, or microbial contamination (Chankana et al., 2024).

3.3 Experimental Design and Solubilization Characteristics Study

The study employed an I-optimal design, varying the mass fractions of three solvents: ethanol, PG, and water, as constraints shown in Equations 1-4 and mass fractions shown in Table 1. For each experimental run (n = 3), 100 mg of cannabis extract was added to a 2-mL microcentrifuge tube, followed by 1 g of the premixed solvent mixture. The mixture was then ultrasonicated for 20 min, vortexed for 2 min, and centrifuged at 6,000 rpm for 2 min. The physical appearance of the mixture was recorded using a coded scale (1 to 4): 1 = clear with no residue, 2 = clear with some residue at the bottom, 3 = turbid with some residue at the bottom, and 4 = turbid with insoluble mass at the top.

$$0 \leq A \leq 1 \quad \text{Eq.1}$$

$$0 \leq B \leq 1 \quad \text{Eq.2}$$

$$0 \leq C \leq 0.2 \quad \text{Eq.3}$$

$$A + B + C = 1 \quad \text{Eq.4}$$

Where A, B, and C represent the mass fractions of ethanol, PG, and water, respectively.

The supernatant was collected, weighed to 500 mg, and adjusted with methanol to 10 mL in a volumetric flask. It was further diluted (20×), filtered, and analyzed CBD and Δ^9-THC by HPLC using the method reported previously. The content of CBD and Δ^9-THC was analyzed using an HPLC system (Agilent 1260 Infinity, Agilent Technologies, USA). A C18-PFP column (150×3.0 mm, i.d., 3 μm) (Advanced Chromatography Technologies Ltd., UK) was employed and maintained at 25ºC. The isocratic elution used a mixture of water and methanol (17:83 v/v) at a flow rate of 0.4 mL/min. The injection volume was 5 μL, and detection was carried out at a wavelength of 222 nm. This method has already been validated to ensure its reliability (Yangsud et al., 2021b). CBD and Δ^9-THC recoveries were calculated aby comparing them to their respective contents in the cannabis extract.

Data were analyzed by the Design-Expert® v. 11 software. A contour plot and trace (Piepel) plot were created. Analysis of variance (ANOVA) was mentioned. The solvent system that produced a clear solution with maximum CBD and Δ^9-THC recovery was selected as an optimized solvent system to verify prediction accuracy and reliability based on percentage error.

Table 1 The I-optimal design varied the mass fractions of ethanol, PG, and water, along with their corresponding responses.

Std	Run	Mass fraction			Response		
		Ethanol	PG	Water	Soluble characteristic*	CBD recovery	Δ^9-THC recovery
3	1	0.33	0.67	0.00	1	97.66 ± 7.15	97.32 ± 7.36
9	2	0.42	0.47	0.11	2	98.02 ± 4.65	96.76 ± 4.58
8	3	0.95	0.00	0.05	1	100.39 ± 6.63	100.08 ± 6.74
7	4	0.95	0.00	0.05	1	102.48 ± 9.79	102.18 ± 9.51
6	5	0.00	0.96	0.04	4	93.36 ± 1.35	85.77 ± 2.82
10	6	0.42	0.47	0.11	2	94.49 ± 8.77	93.03 ± 9.16
13	7	0.57	0.23	0.20	2	92.62 ± 1.86	90.01 ± 1.96
11	8	0.42	0.47	0.11	2	92.75 ± 2.28	91.23 ± 2.75
14	9	0.21	0.59	0.20	3	64.71 ± 5.17	44.31 ± 3.42
2	10	0.65	0.35	0.00	2	93.60 ± 4.19	92.82 ± 4.23
12	11	0.42	0.47	0.11	2	96.18 ± 1.01	94.63 ± 1.12
5	12	0.00	0.96	0.04	4	94.45 ± 2.69	88.94 ± 3.50
4	13	0.17	0.83	0.00	1	96.16 ± 7.45	95.66 ± 7.26
16	14	0.78	0.02	0.20	2	97.88 ± 2.63	97.19 ± 2.70
1	15	0.81	0.19	0.00	1	98.16 ± 2.22	97.70 ± 2.15
15	16	0.04	0.76	0.20	3	41.26 ± 5.38	25.11 ± 5.48

* 1 = clear with no residue, 2 = clear with some residue at the bottom, 3 = turbid with some residue at the bottom, and 4 = turbid with insoluble mass at the top.

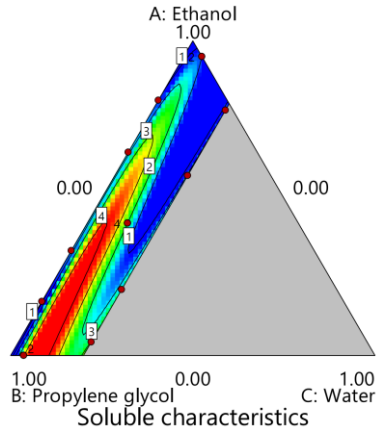

Figure 1 Contour plot of soluble characteristics of the cannabis extract in various solvent mixtures.

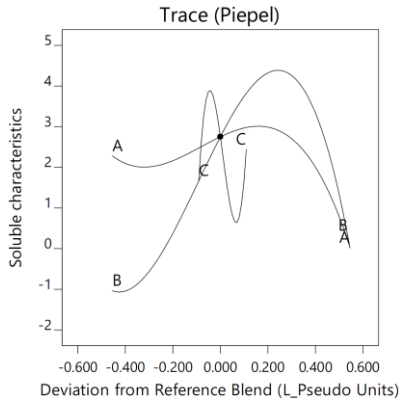

Figure 2 Trace (Piepel) plot of soluble characteristics of the cannabis extract in various solvent mixtures. A, B, and C are mass fractions of ethanol, PG, and water, respectively

4. Results

The qualitative and quantitative responses, including soluble characteristics, CBD recovery, and Δ^9-THC recovery—for individual solvent mixtures are presented in Table 1. The solubilization characteristics of cannabis extract in various solvent mixtures are illustrated in Figure 1, in which the blue area indicates the desired region of complete dissolution. The trace (Piepel) plot in Figure 2 reveals notable trends. Increasing ethanol content enhances cannabis solubility, then decreases solubilization, and ultimately promotes complete dissolution at high concentrations. Similarly, increasing PG initially results in insoluble mixtures; however, at high concentrations, PG surprisingly promotes complete solubility. Water has a more complex effect, initially producing insoluble mixtures, followed by soluble mixtures, and eventually reducing solubilization at high concentrations.

The cannabis extract obtained through supercritical carbon dioxide fluid extraction used in this work contained 14.09 ± 0.72% CBD and 34.19 ± 1.73% Δ^9-THC. These values were used to calculate the recovery of both CBD and Δ^9-THC in the solvent mixture. Figure 3 illustrates the recoveries of CBD and Δ^9-THC in various solvent mixtures. The red area in the contour plots indicates the desired region of high recoveries. Figs. 4a and 4b illustrate the trace (Piepel) plots, highlighting key trends in CBD and Δ^9-THC recoveries. Increasing ethanol content initially appears to boost CBD and Δ^9-THC recoveries, followed by a decrease. Similarly, increasing PG content initially increases CBD and Δ^9-THC recoveries, then decreases them, before unexpectedly enhancing recoveries again at high concentrations. Water has a simpler effect, initially increasing recovery before reducing it. Table 2 indicates that the linear mixture term, as well as interactions between ethanol and water and among all three solvents, were significant for CBD recovery. Table 3 reveals that only the linear mixture term and a specific quadratic term (A^2BC) were significant for Δ^9-THC recovery. Moreover, the lack of fit for both CBD and Δ^9-THC recoveries was not statistically significant, confirming that the observed values closely matched to the predicted values. In other words, the predictive model exhibited high accuracy in forecasting the observed data.

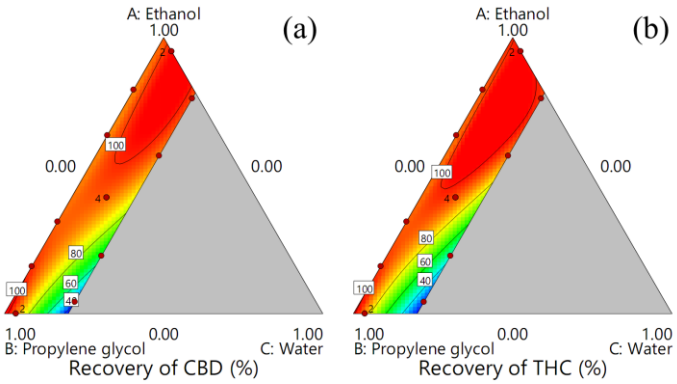

Figure 3 Contour plots of recovery of (a) CBD and (b) Δ^9-THC in various solvent mixtures

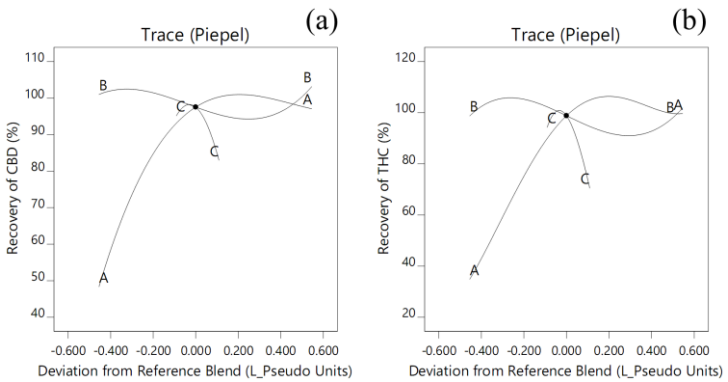

Figure 4 Trace (Piepel) plots of recovery of (a) CBD and (b) Δ^9-THC in various solvent mixtures. A, B, and C are mass fractions of ethanol, PG, and water, respectively.

Table 2 ANOVA for recovery of CBD

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	3628.14	6	604.69	120.60	< 0.0001*
Linear Mixture	1891.46	2	945.73	188.62	< 0.0001*
AB	13.51	1	13.51	2.70	0.1351
AC	73.82	1	73.82	14.72	0.0040*
BC	13.90	1	13.90	2.77	0.1303
ABC	87.69	1	87.69	17.49	0.0024*
Residual	45.13	9	5.01		
Lack of Fit	27.02	4	6.75	1.87	0.2546
Pure Error	18.11	5	3.62		
Cor Total	3673.27	15			

A is the mass fraction of ethanol, B is the mass fraction of PG, C is the mass fraction of water

An asterisk (*) denotes significant value ($p < 0.05$)

Table 3 ANOVA for recovery of Δ^9-THC

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	6672.23	8	834.03	151.83	< 0.0001*
Linear Mixture	3691.72	2	1845.86	336.02	< 0.0001*
AB	4.28	1	4.28	0.7794	0.4066
AC	1.60	1	1.60	0.2905	0.6066
BC	0.1421	1	0.1421	0.0259	0.8768
A^2BC	38.01	1	38.01	6.92	0.0339*
AB^2C	7.06	1	7.06	1.29	0.2943
ABC^2	12.06	1	12.06	2.19	0.1820
Residual	38.45	7	5.49		
Lack of Fit	14.62	2	7.31	1.53	0.3026
Pure Error	23.84	5	4.77		
Cor Total	6710.68	15			

A is the mass fraction of ethanol, B is the mass fraction of PG, and C is the mass fraction of water

An asterisk (*) denotes significant value ($p < 0.05$)

Table 4 Verification data include predicted values, actual values, and percent error

Responses	Predicted values	Batches	Actual values	Error (%)*
Recovery of CBD (%)	98.88	1	97.30 ± 1.41	−1.62
		2	97.93 ± 1.20	−0.97
		3	96.10 ± 1.20	−2.89
Recovery of Δ^9-THC (%)	100.72	1	97.23 ± 1.53	−3.59
		2	97.95 ± 1.22	−2.83
		3	95.35 ± 1.38	−5.63

* Error (%) = (Actual value-Predicted value)×100/Actual value

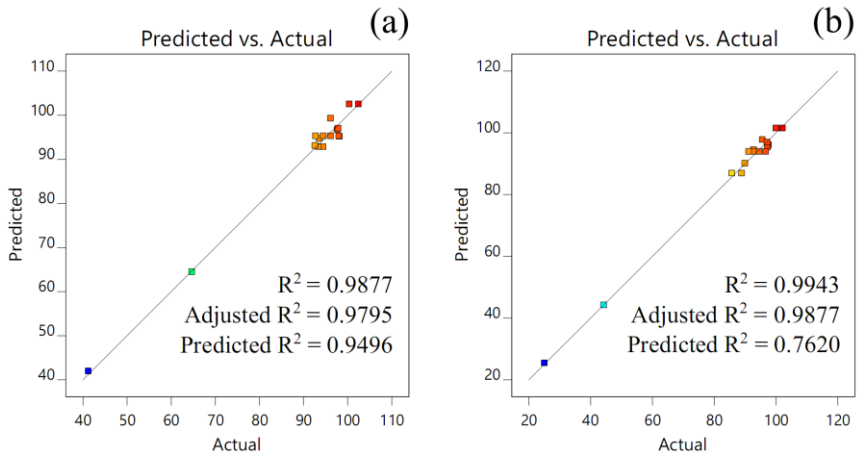

Figure 5 Correlation between predicted and actual values for the recoveries of (a) CBD and (b) Δ^9-THC.

When the mass fraction of water was maximized, the optimized solvent mixture that yielded a clear solution with maximum recoveries of CBD and Δ^9-THC consisted of mass fractions of 0.53 ethanol, 0.35 PG, and 0.12 water. This mixture achieved a desirability value of 0.867. Table 4 presents the percent error between predicted and actual recovery values, further validating the accuracy and reliability of the computer software's predictions. Verification data confirmed the accuracy and reliability of the computer software's prediction, as indicated by the low percentage error of less than 10%. Interestingly, the percentage error for CBD recovery (−0.97 to −2.89) was similarly low to that of Δ^9-THC (−2.83 to −5.63). These low percent error could be explained by the high R^2 values between actual and predicted values: 0.9877 for CBD recovery and 0.9943 for Δ^9-THC recovery (Figure 5).

5. Discussion

CBD and Δ^9-THC are classified as Class II drugs under the Biopharmaceutics Classification System (BCS), which are characterized by poor water solubility but high permeability (Grifoni et al., 2022). Although they have been successfully formulated into the oral spray Sativex®, solubilizing these cannabinoids in liquid formulations remains challenging. High ethanol concentrations, which are often required to dissolve CBD and Δ^9-THC, can cause oromucosal irritation, while high water content results in insoluble mixtures. Ethanol and certain polyols, such as glycerin and PG, are commonly used as solvents for cannabinoids due to their ability to effectively solubilize CBD and Δ^9-THC, as demonstrated in previous studies (Cayman Chemical, 2015; Grifoni et al., 2022; Mannila et al., 2007; Monton et al., 2022; Monton et al., 2019; World Health Organization, 2018; Yangsud et al., 2021a). Consequently, ethanol is typically required in formulations to ensure the solubility of these lipophilic compounds.

The use of water, despite its poor solubility for CBD and Δ^9-THC, was considered in this formulation strategy because of its excellent safety profile and cost-effectiveness. Incorporating water into the solvent system could reduce the overall production costs, making it a highly desirable component. Additionally, the inclusion of PG was based on its established role as a co-solvent in Sativex® (GW Pharma, 2024), which has demonstrated compatibility with CBD and Δ^9-THC, further supporting its use in cannabinoid formulations.

This work aimed to address the challenge of enhancing the solubility of cannabis extracts by applying a Design of Experiments approach, specifically utilizing an I-optimal mixture design. The primary objective was to clarify the solubilization characteristics of cannabis extract, obtained through supercritical carbon dioxide extraction, in various solvent mixtures consisting of ethanol, PG, and water. Adding PG and water to ethanol was expected to achieve two objectives: reducing ethanol content to minimize potential oromucosal irritation and utilizing the safety and cost-effectiveness of water. However, high water content unexpectedly led to an insoluble mixture due to the poor water solubility of CBD and Δ^9-THC. It was hypothesized that the combination of water and PG would maintain an optimal solubilization environment for the cannabinoids while reducing ethanol usage. However,

when water content exceeded certain thresholds, an unexpected outcome emerged—high water levels resulted in an insoluble mixture. This was attributed to the poor water solubility of CBD and Δ^9-THC, as previously reported (Cayman Chemical, 2015; Grifoni et al., 2022; Mannila et al., 2007; Monton et al., 2024c; World Health Organization, 2018). Since these cannabinoids are lipophilic, excess water destabilized the solvent system, leading to precipitation or phase separation.

The solubilization characteristics of cannabis extract in various solvent mixtures were evaluated to ensure an optimal physical appearance of the formulation. In this context, solubilization not only directly reflects the ability of the system to dissolve the extract but also indirectly indicates the recovery of the bioactive compounds, such as CBD and Δ^9-THC. Achieving a clear solution is essential for demonstrating good solubilization, as turbidity can negatively affect the perceived quality of the product from the consumer's perspective (Abdel-Rahman, & Floeter, 2016). The findings indicated that several solvent mixtures successfully produced a clear solution, demonstrating effective solubilization of the cannabis extract. However, this qualitative observation required further validation, so quantitative data on the recoveries of CBD and Δ^9-THC were also investigated to confirm the actual solubility of the cannabis extract.

As mentioned earlier, ethanol and PG effectively solubilize both CBD and Δ^9-THC, as well as the cannabis extract. Using a higher mass fraction of ethanol resulted in better recoveries of CBD and Δ^9-THC, indicating superior solubilization of the cannabis extract. While higher PG concentrations also enhanced the recovery of these compounds, the performance was slightly lower than that of ethanol. This difference may be attributed to the higher viscosity of PG, which can slow the solubility rate relative to ethanol. The impact of viscosity on drug dissolution has been well-documented in prior research (Hassan, & Hasary, 2023).

Interestingly, the use of water in the solvent mixture did not uniformly decrease the recoveries of CBD and Δ^9-THC. In fact, in certain mass fractions, the addition of water was observed to improve the recoveries of these cannabinoids. This phenomenon aligns with findings from previous study, where the inclusion of water enhanced the extraction efficiency of CBD and Δ^9-THC from cultivars like Charlotte's Angel and Hang Kra Rog Phu Phan (Monton et al., 2024a). However, an excessive amount of water eventually diminished the recoveries. The variation in solvent properties across different mass fractions, such as changes in melting and boiling points, dielectric constant, viscosity, and polarity, likely explains these consequences (Lazarjani et al., 2021; Moreno-Sanz et al., 2020; Tzimas et al., 2024). Explaining the effects of the physicochemical properties of individual solvent mixtures is challenging, as the present study did not evaluate these properties directly. Expanding the research to determine the influence of solvent properties on solubility characteristics, CBD recovery, and Δ^9-THC recovery would be valuable to fully understand the relationship between solvent properties and cannabinoid, as well as cannabis extract solubility. To the best of our knowledge, viscosity, polarity, and dielectric constant are particularly critical factors influencing solubilization. Among the solvents used, PG had the highest viscosity compared to ethanol and water, which corresponded to its lower impact on CBD and Δ^9-THC recoveries, as seen in the trace (Piepel) plots (Figure 4). Water, having the highest polarity and dielectric constant, showed significant effects on CBD and Δ^9-THC recoveries with changes in its mass fraction. Ethanol, with a polarity that may be close to that of cannabinoids, facilitated easier cannabinoid dissolution. This is reflected in the trace (Piepel) plots (Figure 4), where an increase in the mass fraction of ethanol enhanced CBD and Δ^9-THC recovery, even when mixed with other solvents. The authors note that future work could be improved by adjusting the polarity of the solvent mixture to match the polarity of ethanol, which might facilitate easier dissolution of cannabinoids.

Despite this challenge, the optimized co-solvent system of ethanol, PG, and a controlled amount of water proved to be a robust and effective alternative for enhancing cannabis extract solubility. The results demonstrated that this ternary solvent system significantly improved the recovery of both CBD and Δ^9-THC, achieving near 100% recovery. Furthermore, the final solutions were clear, with no visible residue or precipitation, indicating that the system effectively maintained cannabinoids in a solubilized state. These findings underscore the potential utility of ethanol-based co-solvent systems in pharmaceutical formulations, particularly in applications where the solubility and stability of cannabinoids are crucial. The enhanced solubility offered by this system could facilitate the development of more effective cannabinoid-based therapies. However, to fully assess its suitability for long-term use, a comprehensive stability study is required. This study would need to evaluate the

stability of both the cannabis extract and its bioactive compounds, such as CBD and Δ^9-THC, within the optimized solvent system over extended periods. Such research is essential to ensure that the formulation maintains its quality, safety, and efficacy throughout its shelf life.

6. Conclusion

The investigation addressed finding a suitable solvent mixture capable of dissolving cannabis extract obtained through supercritical carbon dioxide fluid extraction. This work systematically evaluated the solubilization characteristics of cannabis extract in various solvent mixtures that are commonly used in oral spray formulations. The selection and variation of three solvents—ethanol, PG, and water—were performed through an I-optimal design. The findings demonstrated the efficiency of the solvent mixture in promoting a clear solution, optimizing the recovery of both CBD and Δ^9-THC. Specifically, the optimal solvent composition comprised of 53% ethanol, 35% PG, and 12% water. This condition promoted a clear solution with a complete recovery of both CBD and Δ^9-THC. This optimized solvent mixture was verified, and a low percentage error of less than 10% was achieved. This data proved the accuracy and reliability of the predictions created by the computer software employed in this work. In conclusion, the results of this research clarified the influence of solvent mixtures on the solubilization characteristics of cannabis extract. The identified optimized solvent mixture, with its precise composition, holds significant promise for advancing the development of a cannabis-based oral spray, potentially offering innovative solutions for the treatment of various ailments.

7. Acknowledgements

We extend our gratitude to Ms. Kanyanut Suksumran and Mr. Kritsakorn Ubonsin for their valuable assistance in this research. We also acknowledge the Research Institute of Rangsit University for providing funding under grant number 99/2562. During the preparation of this work the authors used ChatGPT (GPT-4o mini) and Gemini in order to proofread and correct grammatical errors during the manuscript writing process. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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