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Evaluation of compatibility of FluidCrystal® formulations with fibre-type hemp (*Cannabis sativa* L.) extracts

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The main objective of the present study was to investigate mixtures of soy phosphatidylcholine (SPC) and glycerol dioleate (GDO) as encapsulation matrices for *Cannabis sativa* L. phytocannabinoids. The effects of cannabinoids loading into non-aqueous formulations and non-lamellar liquid crystalline phases were studied using synchrotron small-angle X-ray diffraction and dynamic light scattering methods. The incorporation of phytocannabinoids is discussed with respect to the lipid aggregation behaviour, self-assembled nanostructures, and long-term chemical stability. The obtained results showed that SPC/GDO-based formulations can incorporate relatively high amounts of cannabinoids and could serve as liquid crystalline delivery vehicles in the form of bulk phases.

Keywords: phytocannabinoids, *Cannabis sativa*, lipid liquid crystals, lipid-based delivery systems, small-angle X-ray diffraction, antioxidant

INTRODUCTION

Cannabis can be regarded as the most controversial plant in our society [1]. It is widely known for its bioactive constituents, particularly a unique class of terpenophenolic compounds termed cannabinoids or phytocannabinoids [2]. Fibre-type *Cannabis sativa* L., also known as industrial hemp, is an important source of valuable non-psychoactive phytocannabinoids, predominantly cannabidiolic acid (CBDA) and cannabidiol (CBD) [2]. This cannabis has a deficient level of psychoactive Δ^9-tetrahydrocannabinol (Δ^9-THC) [3]. CBDA and CBD have demonstrated various pharmacological activities, including antioxidant, anti-inflammatory, anticonvulsant, anxiolytic and antipsychotic effects, and are currently being tested in clinical trials [2, 4]. Cannabidiol has a therapeutic impact

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on neurodegenerative conditions, pain management, cerebral ischemia-reperfusion injury, heart diseases, vascular diseases, diabetes and hypertension [5]. CBDA, the biogenetic precursor of CBD, is associated with potent health-promoting attributes, including anti-inflammatory, antinausea and anticonvulsant activities [6].

In November 2020, the European Court of Justice published a judgement stating that cannabidiol (CBD) extracted from the cannabis plant should not be considered a drug under the 1961 United Nations Single Convention on Narcotic Drugs [7]. Upon the change in legal regulation, the interest in industrial hemp has initiated new scientific research goals worldwide for its therapeutic, nutraceutical and food applications [6]. Up to now, CBD is available in many formulations (e.g. purified CBD, mixture CBD:THC at different ratios, CBD enriched products) and forms, like oil solution, sublingual tablets, capsules, tablets, sublingual spray, nasal spray, and creams, and can be found in dietary supplements, cosmetics, and animal health products, with Epidiolex® being the first and only FDA-approved CBD-containing drug [5].

In this study, we focused on developing of lipid formulations and non-lamellar LC phases as incorporation matrices for phytocannabinoids extracted from fibre-type cannabis. LCs are typically composed of amphiphilic lipid molecules with polar head groups and nonpolar tails. A wide range of structures such as lamellar, hexagonally arranged channels, and reverse micellar bicontinuous networks can be formed. Hydrated mixtures of soy phosphatidylcholine (SPC) and glycerol dioleate (GDO) form several different lipid liquid crystal (LLC) structures that can incorporate drug molecules and facilitate their release into the surrounding aqueous environment, depending on the lipid composition and temperature. This makes them highly attractive as hosts for biologically active molecules [8–10]. These systems have been extensively studied for their ability to release active pharmaceutical ingredients over long periods of time. They also have the potential to improve the bioavailability of both hydrophilic and hydrophobic drugs. Additionally, their biodegradable, non-toxic and bioadhesive properties contribute to their applications in drug delivery [11–13]. Likewise, accuracy in dose control, reduction in dosing frequency, and improved overall patient compliance lower toxicity-mediated side effects and increase drug efficiency [14]. Characterisation of LC systems is fundamentally important to achieve the goal of controlling their function and enhancing our understanding of their drug release behaviour.

The goal of this study was to investigate SPC/GDO-based lipid formulations that can form non-lamellar liquid crystalline (LC) phases, particularly in relation to their ability to incorporate phytocannabinoids. The structural impact of cannabinoids on these LC phases was analysed through synchrotron small-angle X-ray scattering (SAXS). Chemical stability of the cannabinoids within the lipid matrix was assessed using HPLC-MS, and the DPPH assay was conducted to evaluate the antioxidant potential of the encapsulated compounds.

EXPERIMENTAL

Materials

The cannabidiol (CBD) analytical standard (Product No. C6395, MDL No. MFCD00869597) and the cannabidiolic acid (CBDA) primary measurement standard (Product No. C-144-1ml, Lot. No. FE04301903) were purchased from Sigma-Aldrich (Saint Louis, USA). Glycerol dioleate (HP GDO from Croda, Staffordshire, United Kingdom), containing diglycerides (>96.0%), 1,2-propylene glycol (PG) from Scharlau (Barcelona, Spain), and soybean phosphatidylcholine (S100 from Lipoid GmbH, Ludwigshafen, Germany), with the major components: phosphatidylcholine (>97.0%) lysophosphatidylcholine (<1.0%), triglycerides (<2.0%), and free fatty acid (<0.05%). Methanol (MeOH), hexane, formic acid (FA), and acetonitrile (ACN) were of HPLC grade and obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), and ethanol absolute (99.5%) from Merck KGaA (Darmstadt, Germany). DPPH (2,2-diphenyl-1-picrylhydrazyl) (CAS-No. 1898-66-4) and Trolox ((±)6-hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid) (CAS-No. 53188-07-1) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The water used in this study was cleaned using a Millipore Direct Q water purification system (Merck KGaA, Darmstadt, Germany). Samples of fibre-type *Cannabis sativa* L. plants of 'Futura 75' culture were collected in one field in Vilnius City District (Lithuania) during flowering from 2018 to 2020.

Sample preparation and decarboxylation reactions of *Cannabis sativa* extract

The collected hemp leaves were dried at room temperature for two weeks. Crumbly samples were ground using a Retsch SM100 cutting mill (Retsch GmbH, Haan, Germany). 15 g of powdered leaves sample was extracted with 100 mL of hexane by agitation in an ultrasonic bath for 45 min (*repeated twice*). Following filtration, both extracts were dried at room temperature under a nitrogen stream. The dry residues were then redissolved in 10 mL of absolute ethanol (one-tenth of the solvent volume), filtred once more, and evaporated to dryness under nitrogen, yielding what was termed the phytocannabinoid raw extract (PCRE).

For the decarboxylation reactions, separate vials containing 100 mg/mL of extracts dissolved in EtOH were dried in a Memmert EXCELLENT UNE 400 oven (Memmert GmbH, Schwabach, Germany) at 160°C for 40 min. After decarboxylation, the extracts were cooled to room temperature. This extract was denoted as the phytocannabinoid extract after the decarboxylation (PCEAD). Each extract was divided into two portions. One portion was re-dissolved in EtOH to obtain a 10 mg/mL concentration for HPLC/DAD-MS analysis (n = 3). Another portion was used to prepare lipid-based formulations.

Preparation of lipid-based formulations

Non-aqueous lipid formulations were prepared by mixing appropriate amounts of lipid components (SPC and GDO) in the presence of 5% PG and 10 wt% ethanol to facilitate mixing. The lipid mixtures were then placed on a roller mixer at room temperature (RT) for 24 h until thoroughly mixed. The prepared lipid formulations were stored at room temperature until further use. *Cannabis sativa* phytocannabinoids containing formulations were prepared by adding an appropriate amount of dry phytocannabinoids extract to a non-aqueous lipid formulation and placing it on a roller mixer for 48–60 h at RT until a homogeneous mixture was obtained. Visual inspection of the cross-polariser and light microscopy was used to inspect the samples for the presence of undissolved compounds. In this study, the concentration of cannabinoids was expressed as wt% of the total formulation weight.

HPLC/DAD and HPLC-MS analysis

The LC-DAD separation was achieved using a Zorbax Eclipse XDB–C18 (5 μm, 150 × 4.6 mm) column (Agilent Technologies, Santa Clara, CA, USA) coupled with a Zorbax Eclipse XDB–C18 (5 μm, 12.5 × 4.6 mm) guard column (Agilent Technologies, Santa Clara, CA, USA) on an Agilent 1260 Infinity Liquid Chromatography system (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump, a vacuum degasser module, an autosampler, a column compartment (maintained at 35°C) and a diode array detector (DAD) set at 220 nm. Separations were carried out in a binary solvent system: solvent A, 0.1% formic acid in water; and solvent B, 0.1% formic acid in acetonitrile. The initial setting was 60% B (v/v), which was linearly increased to 90% B over 29 min and then decreased to 60% in 4 min. This condition was maintained for 2 min. The flow-rate was set to 0.7 mL/min; the injection volume was 10 μL. The total runtime was 35 min.

Electrospray ionisation (Dual-ESI) in the positive ion detection mode was obtained using an Agilent 6224 Accurate-Mass Time-of-Flight mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Zorbax Eclipse XDB–C18 (5 μm, 150 × 4.6 mm) reversed-phase column and a mobile phase of ACN/FA/H_2O at a 0.7 mL/min flow rate, as described above, were used. The typical parameters were as follows: fragmentor voltage 125 V, skimmer voltage 65 V, capillary voltage 3.0 kV, source gas temperature 325°C, gas (nitrogen) pressure 35 psi and gas flow rate 10 L/min. The mass spectrometer was operated with a full scan (50–800 m/z) to analyse the phytocannabinoids. Molecular ions $[M+H]^+$ for CBD and CBDA were extracted from the full scan chromatograms (i.e. TIC → EIC), and peak areas were integrated using the Agilent MassHunter Quantitative Analysis software, version B.05.00 (Agilent Technologies, Santa Clara, CA, USA).

Selective chromatograms were acquired at 220 nm for both analytes. Determination of the limit of detection (LOD) and the limit of quantitation (LOQ) as well as the linear range for CBD and CBDA were carried out using a series of seven dilutions (ranging from 0.25 to 25 μg/mL). Calibration curves were generated by plotting the relative response to each cannabinoid concentration. Averages of the responses for each standard were

calculated and plotted against the known concentrations. These plots were used for any further calculations, that is, determining the LOD and LOQ, and calculating the CBD and CBDA concentrations of samples (see Table 1).

Table 1. **Quantification parameters of phytocannabinoids**

	Cannabidiolic acid	Cannabidiol
Retention time, min	**11.20 min**	**12.60 min**
LOD, µg/mL	0.25	0.25
LOQ, µg/mL	0.75	0.75
Linearity range, µg/mL	0.75–25.0	0.75–25.0
Linearity, r	0.9999	0.999
Repeatability RSD, %	<7.0	<5.7
Target ion, m/z	359	315
Ion extraction window, m/z	358.5–359.5	314.5–315.5

Phytocannabinoids stability study

The chemical stability of CBD and CBDA entrapped in non-aqueous lipid formulations was monitored for 6 months. During the stability study, all the samples were kept at RT in the dark. To evaluate the residual CBD and CBDA concentrations, a portion of the sample (approximately 10 mg) was collected at predetermined time points (1, 30, 60, 120 and 180 days), dissolved in EtOH at a lipid sample to a solvent weight ratio of 1:10, and analysed using HPLC/DAD and/or HPLC/MS. Each sample was analysed in triplicate.

DPPH radical scavenging activity assay

The DPPH assay was performed according to the method described by Brand-Williams et al. [15], with some modifications. A $6{\cdot}10^{-5}$ M stock solution of DPPH was prepared by dissolving 2,2-diphenyl-1-picrylhydrazyl in methanol. Subsequently, the solution was diluted with methanol to obtain an absorbance of 0.730 ± 0.02 units at 515 nm using a spectrophotometer (UV–Vis LAMBDA 25, PerkinElmer, USA). Standard 10 mm disposable plastic cuvettes were used for the absorbance measurements. A small sample specimen (about 100 mg) was collected and dissolved in 1 mL of ethanol absolute. Then 0.1 mL of the prepared sample was allowed to react with 3.9 mL of working DPPH solution for 30 min in the dark. After the reaction, absorbance values were recorded at 515 nm using a spectrophotometer. Antioxidant activity was evaluated using a standard Trolox curve in the 25 and 200 mM concentration range. The results are expressed in mM of Trolox equivalents (TEAC) as a mean result with a standard deviation of the triplicate experimental setup.

5 mg of Trolox was dissolved in methanol and water solution (70:30, v/v) and diluted until 100 mL. Five different concentrations from this solution were prepared (100, 75, 50, 25 and 12.5%). 0.1 mL of each concentration was allowed to react with 3.9 mL of the working solutions of DPPH. Absorbance values were recorded at 515 nm using a spectrophotometer. Linear calibration curves were obtained, and their parameters were used to calculate antioxidant capacity further. All measurements were done in triplicate.

Small angle X-ray scattering analysis

Lipid mixtures of three lipid compositions were prepared, each containing a range of *Cannabis sativa* phytocannabinoid concentrations from 0 to 12.5% (w/w), and analysed using small-angle X-ray scattering (SAXS). Lipid depots of 200 mg were prepared in phosphate-buffered saline (PBS) with pH 7.4 (0.14 M NaCl, 0.0027 M KCl and 0.010 M phosphate buffer) and stored at room temperature (RT). Two sets of depots were prepared for each lipid formulation: in one set, the depot was vortexed immediately after injecting the formulation into the PBS solution. All depots were left to equilibrate at RT for two weeks prior to analysis.

Small-angle X-ray scattering (SAXS) experiments were carried out at a XEUSS 3.0 X-ray scattering instrument with a copper source (1.54 Å). Two-dimensional SAXS images were recorded using a SAXS detector PILATUS 300 K located at a distance of 800 mm. The sample to detector distance was calibrated by using a silver behenate sample. The reported scattering profiles I(q) were obtained by radially averaging the 2D SAXS ages. With this setup, a range of q-values, q = 0.01– 0.36 Å^{-1}, was covered. The samples were sealed at room temperature between two thin Kapton windows in a metallic block or in a 1.5 mm borosilicate capillary, depending on the sample viscosity. All the samples were analysed at 25°C. The background was removed by subtracting the signal from the empty Kapton windows or empty capillaries from the measurements.

RESULTS AND DISCUSSION

Evaluation of extraction technique

Up to now, approximately 566 phytochemicals have already been identified in cannabis plants [1, 16]. More than 125 of these compounds are cannabinoids (phytocannabinoids) [4, 16]. The phytocannabinoids are biosynthesised in an acidic (carboxylated) form in the living plant and/or fresh plant materials. In plant tissues, cannabidiolic acid (CBDA) and analogous cannabinoids are readily decarboxylated to their neutral forms in the presence of heat and during other mechanical manipulation and/or storage [1, 17]. Dried and aged plant material contains both neutral and acidic forms of CBD. In this segment of the study, the goal was to extract fractions with high concentrations of the non-psychoactive compounds CBDA and CBD from fibre-type cannabis. It has been reported [18] that different groups of compounds can be directly separated during extraction by employing techniques, such as dynamic maceration, solid phase extraction (SPE), matrix solid-phase dispersion (MSPD), or ultrasound.

The first step in the extraction process was to choose suitable solvents or their mixtures to isolate natural phytocannabinoids. After pilot experiments with different solvents and their combinations, hexane and absolute ethanol were chosen to extract cannabinoids from dry cannabis. The second step was to evaluate the suitability of the selected solvents for HPLC and/or HPLC-MS analysis. Despite the limited compatibility with reverse-phase HPLC, hexane extracts still offered the best results for isolating CBDA and CBD. Although pure ethanol extracted approximately 90% more compounds than hexane, many of these were chlorophyll-type (see Fig. 1). Hexane also captured some terpenes and additional compounds but proved optimal for cannabinoid isolation. The dry extracts were repeatedly redissolved in absolute ethanol, filtered, evaporated, and the final residuals were used in further studies. In this part of study, we observed the conversion of cannabidiolic acid to neutral cannabidiol (under normal conditions). Therefore, we decided to include an additional decarboxylation procedure using the separated raw extract (PCRE). Figure 2 shows that CBD is the predominant compound in the extracts after decarboxylation (PCEAD). It comprised approximately 67–69% of all compounds extracted according to the full-scan MS chromatogram (i.e. TIC → BPC). Similar results were obtained with UV–VIS detection at 220 nm wavelength.

The selectivity of CBDA and CBD determination in raw extracts (PCRE), extracts after decarboxylation (PCEAD), and SPC/GDO-based non-aqueous formulations was confirmed by comparing retention times, UV spectra, and m/z values to those of analytical standards (Fig. 3).

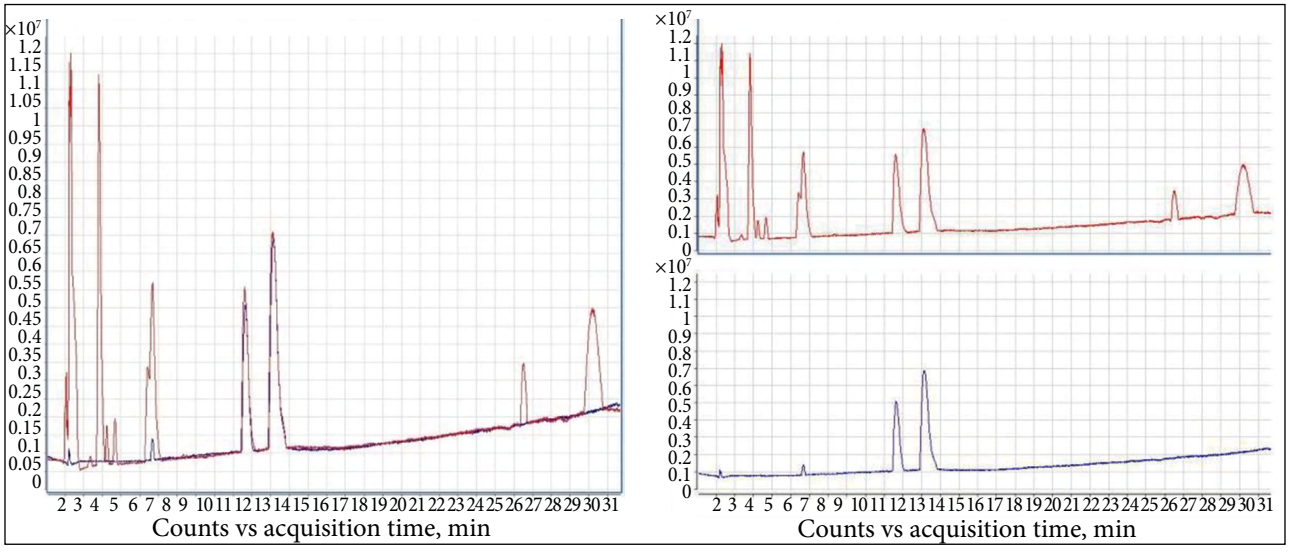

Fig. 1. HPLC-MS chromatograms (TIC → BPC) of pure ethanol extract (red line) and hexane extract (blue line). Detailed conditions of extraction and HPLC-MS procedures are described in the Sections 'Sample preparation and decarboxylation reactions of *Cannabis sativa* extract' and 'HPLC/DAD and HPLC-MS analysis', respectively

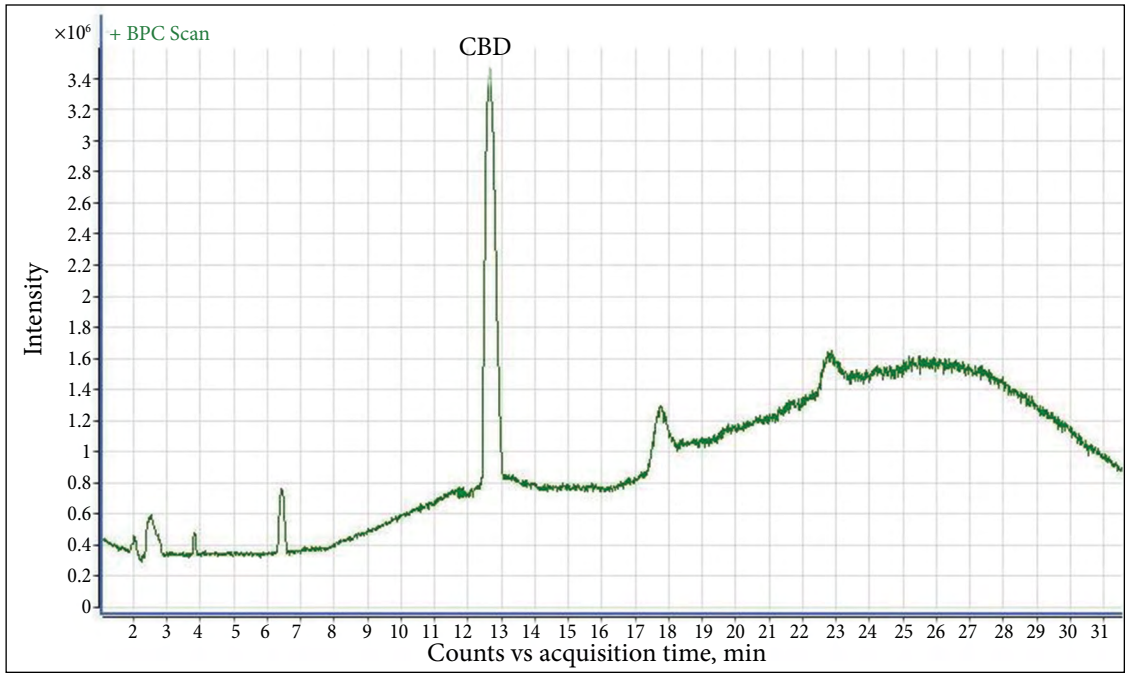

Fig. 2. Typical HPLC-MS chromatogram (TIC → BPC) of fibre-type cannabis extract after decarboxylation (PCEAD). Extraction conditions are described in the Section 'Sample preparation and decarboxylation reactions of *Cannabis sativa* extract'

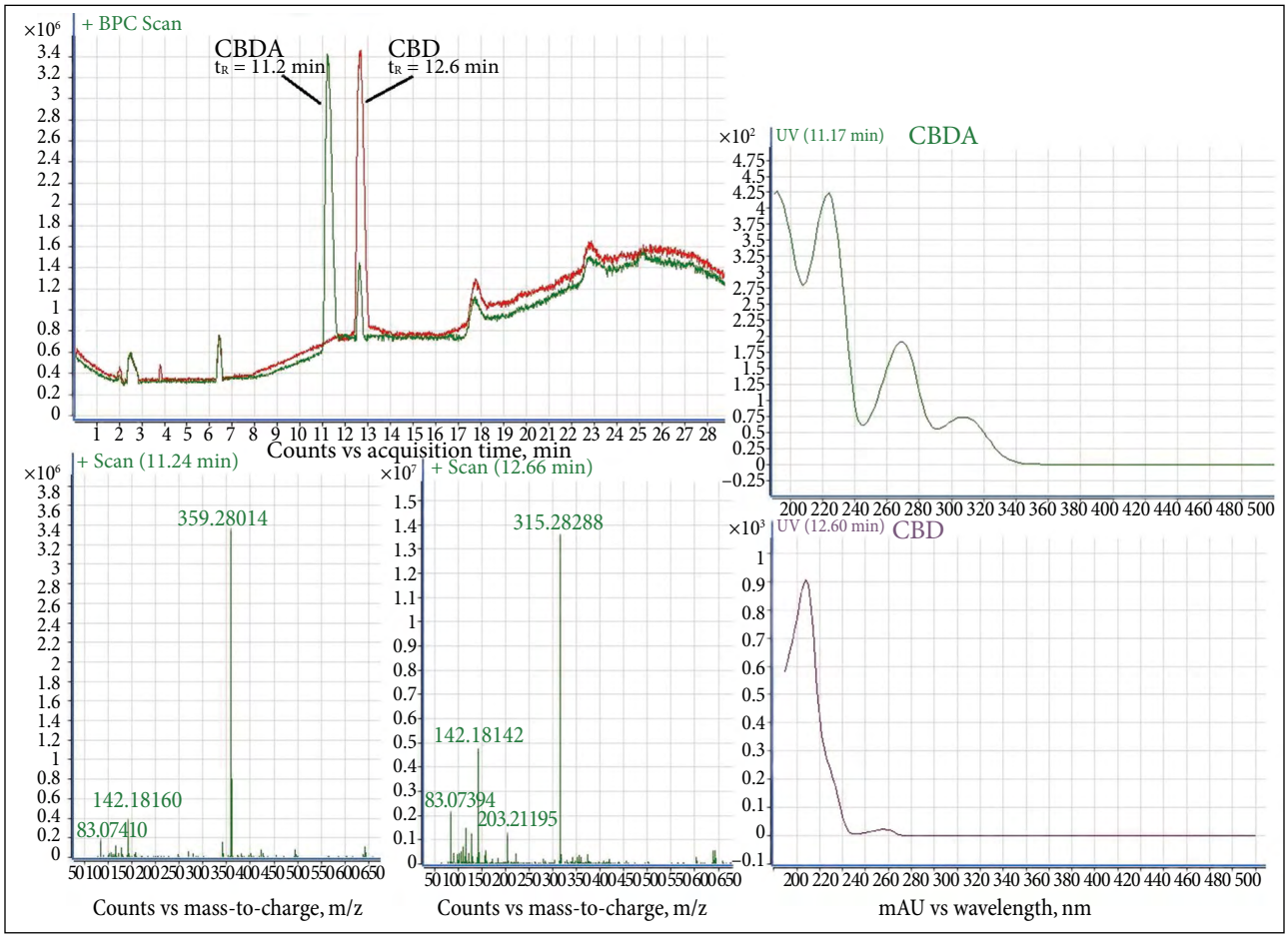

Fig. 3. HPLC-MS chromatograms (TIC → BPC) of *C. sativa* raw extract (green line) and *C. sativa* extract after decarboxylation (a red line). UV–Vis absorbance spectra of CBDA and CBD. Chromatographic conditions as described in the Section 'HPLC/DAD and HPLC-MS analysis

The obtained results of each cannabinoid quantification are listed in Table 1.

Lipid-based delivery systems with phytocannabinoids from fibre-type cannabis

Before further studies, several non-lamellar LC phases that formed non-aqueous lipid mixtures were investigated with respect to their ability to solubilise phytocannabinoids. First, binary lipid mixtures of soy phosphatidylcholine (SPC) and glycerol dioleate (GDO) with and without ethanol were explored. The addition of up to 10 wt% ethanol (with respect to the total lipid ratio) did not improve the solubility of cannabis extracts, that is, PCRE and PCEAD. To maintain the ethanol content in the lipid mixtures at a low level, propylene glycol (PG) was included as an additional co-solvent. Considering the final solubility results, the SPC/GDO-based formulations with 5% PG and 10 wt% ethanol were selected for further experiments.

Small angle X-ray scattering analysis

The impacts of phytocannabinoids on the nanostructure of hydrated SPC/GDO mixtures were the investigated SAXS. Three fully hydrated mixtures of SPC/GDO with increasing concentrations of *Cannabis sativa* phytocannabinoid extract after decarboxylation (PCEAD) were analysed, as shown in Fig. 4.

Figure 4(A) shows the SAXS results for the lipid ratio 35/65 SPC/GDO, and it is evident that even a small amount of PCEAD significantly altered the liquid crystalline structure. Consistent with previous studies [19], the hydrated mixture without PCEAD forms a $Fd\bar{3}m$ structure. However, for the lipid composition of 35/65 SPC/GDO, the addition of PCEAD led to the formation of an isotropic micellar mixture, implying that PCEAD prevents the formation of a cubic phase and induces the formation of a reverse micellar phase, similar to lipid mixtures containing higher concentrations of GDO. Furthermore, the scattering data revealed a slight shift in the peak position to higher q-values with increasing PCEAD concentration, indicating a decrease in the average micelle size. This observation is consistent with the behaviour of GDO-rich mixtures, which have been shown to form smaller micelles [19].

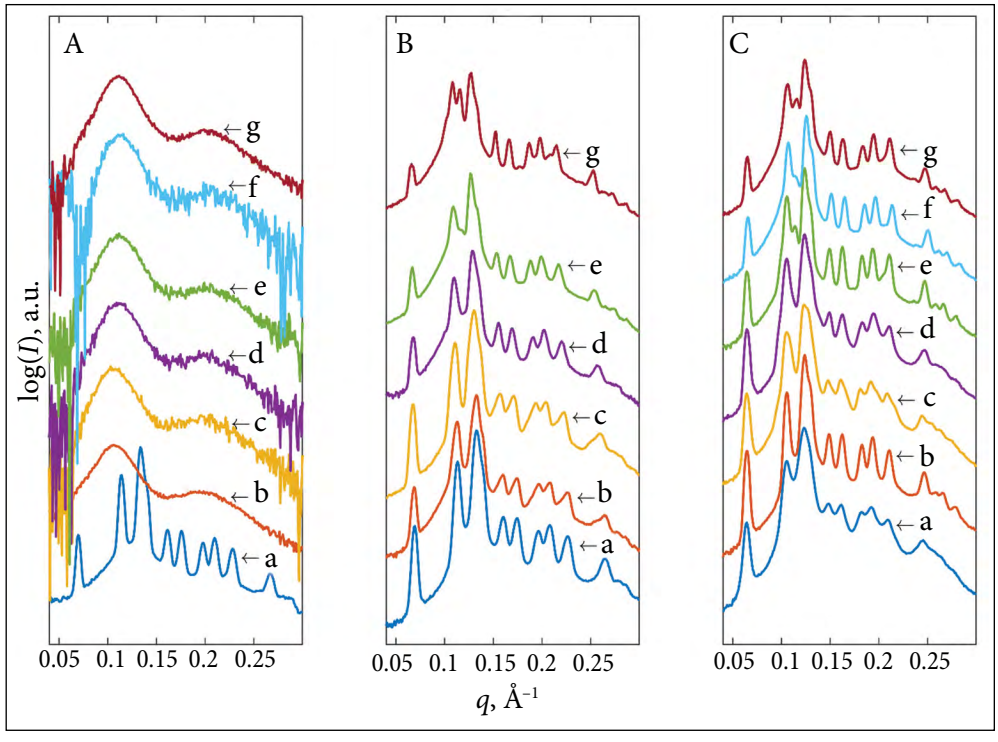

Fig. 4. SAXS results for three hydrated formulations with SPC, GDO, and increasing concentrations of PCEAD: (a) 0% PCEAD, (b) 1% PCEAD, (c) 2.5% PCEAD, (d) 5% PCEAD, (e) 7.5% PCEAD, (f) 10% PCEAD and (g) 12.5% PCEAD. These are colour-coded consequently in each figure. (A) SPC/GDO ratio of 35/65, (B) SPC/GDO ratio of 40/60 and (C) SPC/GDO ratio of 45/55

For mixtures with higher SPC ratios (Figs 4(B, C), for 40/60 and 45/55 SPC/GDO, respectively), no significant changes in the lipid crystalline structure were observed with the addition of PCEAD. Even with 12.5% PCEAD, the characteristic Bragg peaks for $Fd\bar{3}m$, following the 1:√3:√8:√11:√12:√16 ratio, remained intact. Throughout the series, the Bragg peaks consistently followed this ratio. Interestingly, in the blank sample of the 45/55 SPC/GDO mixture, additional Bragg peaks corresponding to a hexagonal phase (with ratios of 1:√3:√4) were also observed, which can be confirmed by polarised light microscopy. However, the overlap of these peaks with those from the $Fd\bar{3}m$ structure makes it challenging to definitively distinguish between the two phases. As the addition of PCEAD alters the colour of the formulation, it becomes increasingly difficult to observe birefringence in polarised light, especially for samples with a higher PCEAD content. Thus, if PCEAD induces a transition from a mixed hexagonal and cubic phase to a purely cubic phase, this change was not definitively observed. However, an additional peak emerged (marked with a red arrow for 40/60 and 45/55 SPC/GDO, respectively) with increasing PCEAD concentration, most noticeably in the samples with the highest PCEAD content. This suggests the presence of a mixed phase, which is not typically observed in pure SPC/GDO mixtures. Additionally, no notable shifts in the peak position were detected, indicating that the unit cell size of the $Fd\bar{3}m$ lattice remains unchanged, implying that PCEAD does not significantly affect the hydration properties of these mixtures.

The samples were stable for two weeks, but some phase separation was observed over time. The PCEAD appeared to migrate toward the centre of the lipid liquid crystalline depot, and over an extended period, the structure exhibited an oily, dark centre, while the outer edges of the depot became clearer.

Phytocannabinoids stability in SPC/GDO non-aqueous formulations

One of the most important aspects of delivery formulations is the chemical stability of the active substances. HPLC was used to evaluate the chemical stability of phytocannabinoids entrapped in the studied SPC/GDO-based systems. The samples were prepared at the single SPC/GDO weight ratio containing 1.0, 2.5, 5.0, 7.5, 10.0 and 12.5 wt% of PCRE's and PCEAD's for the dry formulation weight.

CBD showed an excellent chemical stability for up to 120 days when PCEAD was solubilised in non-aqueous SPC/GDO-based formulations (Fig. 5). Regardless of the concentration of the phytocannabinoid extract, about 94–99% of CBD was retained. Nevertheless, after 6 months, its content in the LC formulations prepared with 1.0, 2.5 and 7.5 wt% by weight extracts after decarboxylation decreased to about 84%.

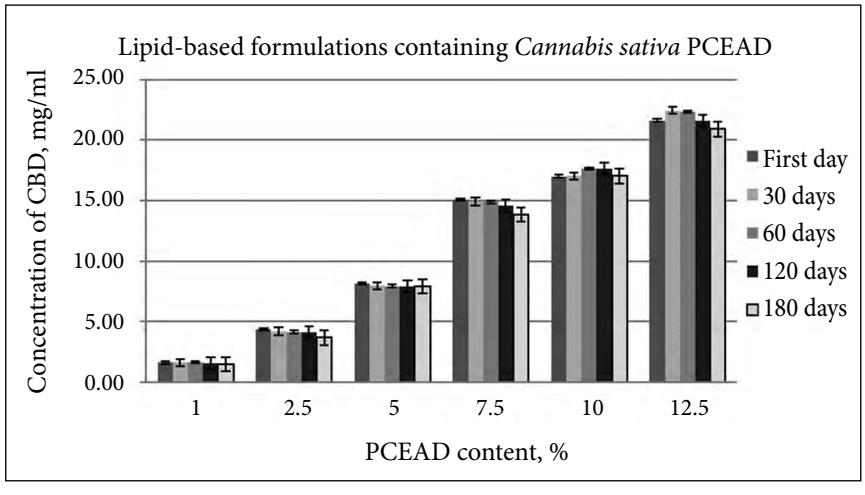

Fig. 5. Chemical stability of CBD in SPC/GDO based non-aqueous formulations. Samples were prepared at a fixed SPC/GDO weight ratio of 35/65 containing 0, 2.5, 5.0, 7.5, 10.0 and 12.5 wt% of PCEAD's with respect to the dry formulation weight. The results are represented as mean values ± standard deviation ($n = 3$)

In raw extracts from fresh plants, the concentration of CBDA was 153.1 mg/g with a 5% deviation. Meanwhile, the concentration of neutral cannabidiol was much lower than cannabidiolic acid and was 39.4 mg/g with a 2% deviation. Figure 3 shows (a green line) that CBDA and CBD comprised approximately 63–65 and 7–9% of the compounds extracted according to the full scan MS chromatogram (i.e. TIC → BPC), respectively.

A gradual and significant loss in the CBDA content upon storage was observed in the PCRE-loaded lipid-based system (Fig. 6), in contrast to the same systems with the entrapped extract after decarboxylation. The CBDA content decreased to 85–87% after 30 days regardless of the phytocannabinoid extract concentration, whereas only 38–43% of CBDA was found at the end of the study. Meanwhile, the CBD concentration increased by 280% (or 112.3 mg/g) compared to the initial content (Fig. 7).

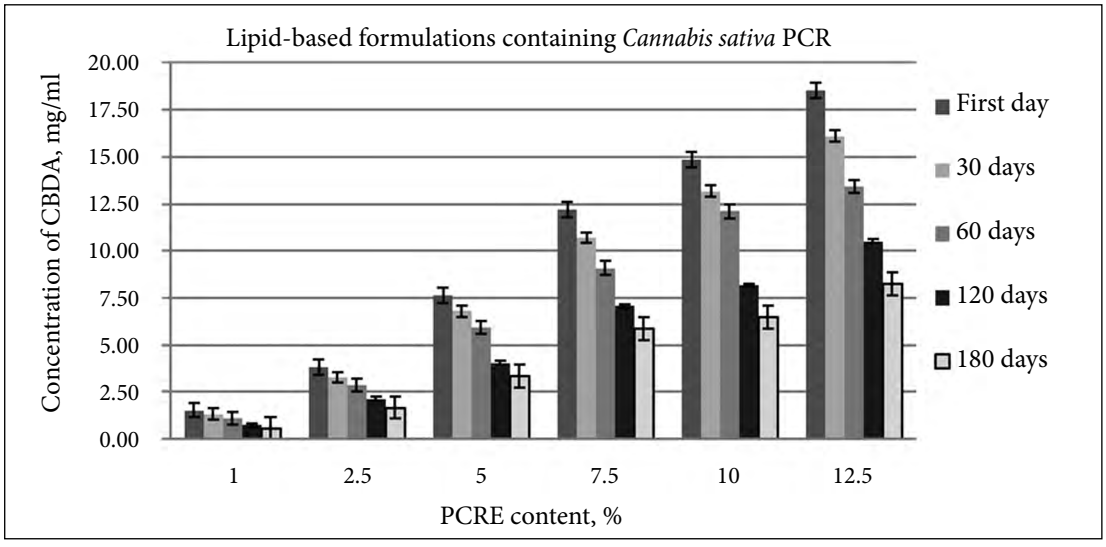

Fig. 6. Chemical stability of CBDA in SPC/GDO based non-aqueous formulations. Samples were prepared at a fixed SPC/GDO weight ratio of 35/65 containing 0, 2.5, 5.0, 7.5, 10.0 and 12.5 wt% of PCRE's with respect to the dry formulation weight. The results are represented as mean values ± standard deviation ($n = 3$)

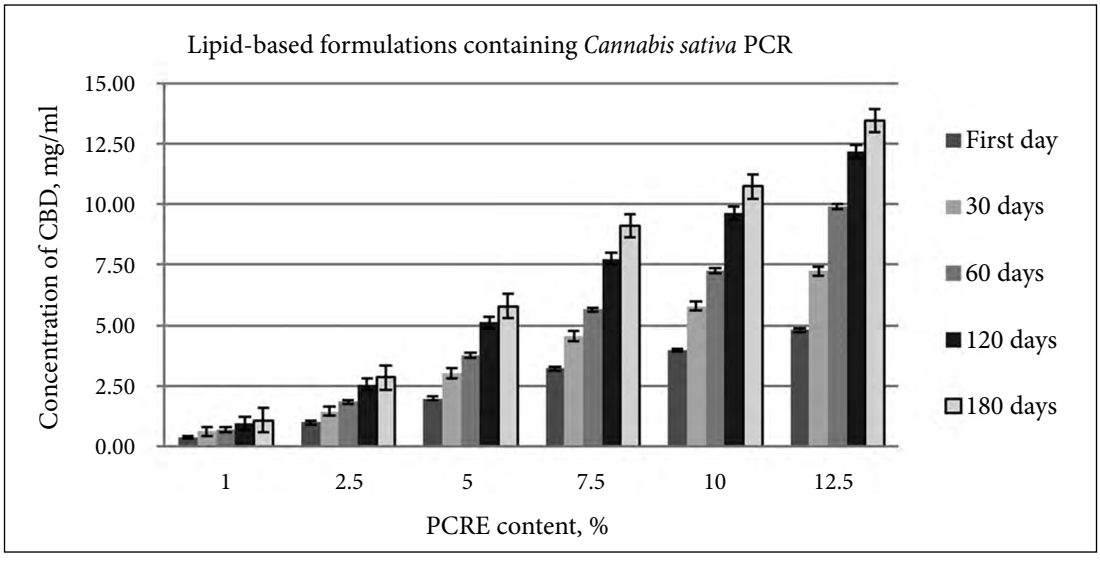

Fig. 7. Chemical stability of CBD in SPC/GDO based non-aqueous formulations. Samples were prepared at a fixed SPC/GDO weight ratio of 35/65 containing 0, 2.5, 5.0, 7.5, 10.0 and 12.5 wt% of PCRE's with respect to the dry formulation weight. The results are represented as mean values ± standard deviation ($n = 3$)

The SPC/GDO-based formulations with entrapped natural bioactive CBDA did not stop the conversion of this compound to CBD over the study period.

Antioxidant activity is often the focal point for monitoring the stability of bioactive compounds. This ensures the efficacy of a product with a specific function [20]. In this part of the study, the free-radical scavenging ability of fibre-type cannabis phytocannabinoids loaded into LC formulations was investigated and compared using the DPPH• assay [15]. Primary screening measurements were performed with freshly harvested dried fibre-hemp, focusing on antioxidant activity (Table 2). Samples were prepared at a fixed SPC/GDO weight ratio of 35/65 containing 2.5 and 12.5 wt% of phytocannabinoids (i.e. PCRE and PCEAD) with respect to the dry weight. Primary PCRE and PCEAD in absolute ethanol (10 mg/mL) were also included in this study.

Table 2. **The antioxidant activity of PCRE and PCEAD in absolute ethanol (1 mg/ml); 2.5 and 12.5% entrapped in non-aqueous SPC/GDO-based formulations. Note: the antioxidant activity (TE) was calculated for 100 mg of the sample. The results are represented as mean values ± standard deviation (*n* = 3)**

	Antioxidation activity, mM (TEAC)	
Type of substance	PCRE	PCEAD
2.5% in LC formulation	1.781 ± 0.026	1.524 ± 0.021
12.5% in LC formulation	6.048 ± 0.004	5.569 ± 0.137
In pure ethanol (1 mg/ml)	5.906 ± 0.015	5.545 ± 0.026

These results indicate that PCRE has higher TEAC properties, regardless of the phytocannabinoid concentration in lipid-based systems (Table 2). CBDA is the predominant compound in these extracts. However, no statistically significant difference was observed between the free radical scavenging abilities of PCRE and PCEAD.

In this study, the conversion of CBDA to neutral CBD was determined already after 30 days (more than 10%). Further detailed antioxidant activity studies were conducted using only PCEAD. For that purpose, samples were prepared at a fixed SPC/GDO weight ratio of 35/65 containing 1.0, 2.5, 5.0, 7.5, 10.0 and 12.5 wt% of PCEAD with respect to the dry weight. On the initial day of the experiment, the free radical scavenging activity for PCAED SPC/GDO-based formulations was TEAC = 1.524 ± 0.021 and TEAC = 5.569 ± 0.137 for formulations containing 2.5 and 12.5% PCEAD, respectively. After 60 days, these values were TEAC = 1.75 ± 0.042 and TEAC = 6.25 ± 0.139, respectively. This insignificant increase of AA can undergo further more detailed investigation. In the 60-day long-term experiment, a positive correlation was consistently observed between the added amount of phytocannabinoids and the free radical scavenging capacity across all tested samples (Fig. 8).

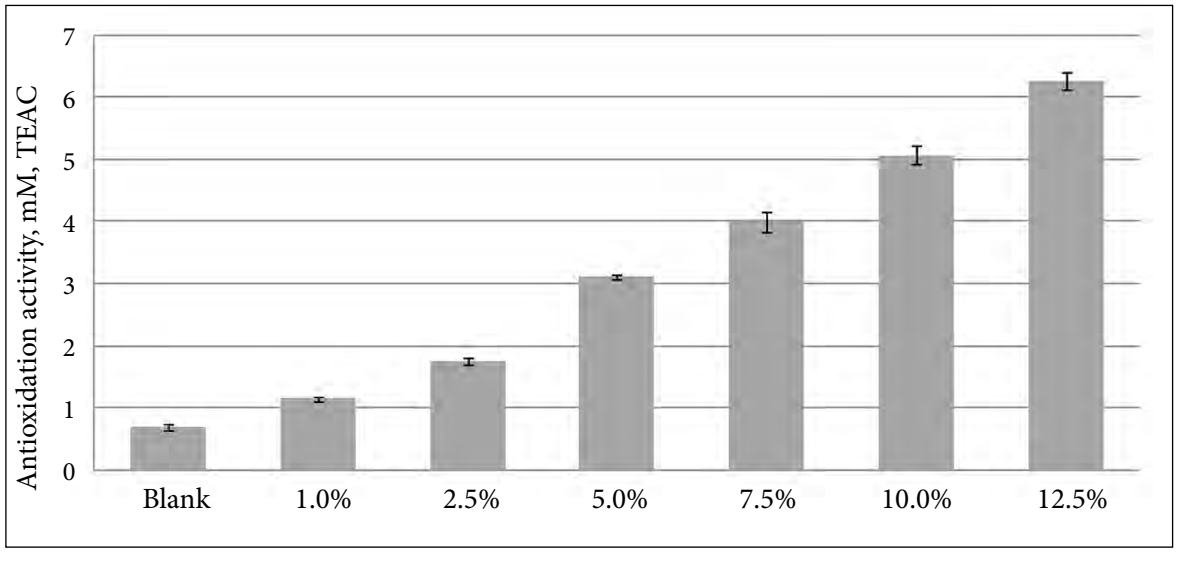

Fig. 8. The antioxidant activity of PCEAD's entrapped in lipid SPC/GDO based non-aqueous lipid formulations. Note: the antioxidant activity (TE) was calculated for 100 mg of the sample. The concentration of active compounds was 1.0, 2.5, 5.0, 7.5, 10.0 and 12.5 wt%. The results are represented as mean values ± standard deviation (*n* = 3)

CONCLUSIONS

The natural phytocannabinoids were successfully extracted from industrial hemp using liquid extraction on dry matter and loaded into lipid SPC/GDO-based delivery systems. The study revealed that the solubility limit of PCRE and PCEAD extracts in non-aqueous SPC/GDO formulations with 10% of ethanol and 5% propylene glycol (PG) was 12.5 wt. The formulations with a higher SPC ratio form the micellar cubic *Fd*$\bar{3}$*m* phase, and this occurs for every concentration of PCEAD. The chemical stability test indicated that non-aqueous SPC/GDO formulations with a high concentration of CBDA did not prevent the gradual conversion of the bioactive compound to the neutral form CBD over time. In contrast, PCEAD-loaded formulations maintained stability after 60 days of storage, preserving their antioxidant properties throughout this period.

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LIPIDŲ SAVITVARKIŲ SKYSTAKRISTALIŲ *FluidCrystal*® SISTEMŲ SU ĮTERPTAIS PLUOŠTINIŲ KANAPIŲ (*CANNABIS SATIVA* L.) FITOKANABINOIDAIS SUDERINAMUMO ĮVERTINIMAS

S a n t r a u k a

Darbe pritaikyta skysčių ekstrakcija iš sausos žaliavos, su tikslu kuo efektyviau išgauti natūralius fitokanabinoidus iš pluoštinės kanapės ir juos įterpti į sojos fosfatidilcholino (SPC) ir glicerolio dioleato (GDO) pagrindu suformuotas lipidines pernašos sistemas. Tyrimai parodė, kad SPC ir GDO pagrindu suformuotose lipidų kompozicijose su 10 % etanolio ir 5 % propilenglikolio (PG) priedu galima ištirpinti iki 12,5 % fitokanabinoidų. Lipidų sistemos su didesniu SPC kiekiu formavo micelinę kubinę *Fd*$\bar{3}$*m* fazę, nepriklausomai nuo įterpto dekarboksilinto kanapių ekstrakto koncentracijos (angl. PCEAD). Cheminio stabilumo studija parodė, jog SPC/GDO pagrindu suformuotos sistemos nesustabdė laipsniško biologiškai aktyvaus CBDA perėjimo į neutralią CBD formą. O lipidinės sistemos su įterptais pramoninių kanapių ekstraktais, kuriuose dominavo CBD, ir po 60 dienų išlaikė cheminį stabilumą; taip pat per šį laikotarpį išsaugojo savo antioksidacines savybes.