Evaluation of amorphous and lipid-based formulation strategies to increase the *in vivo* cannabidiol bioavailability in piglets

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Abstract

Cannabidiol (CBD) suffers from poor oral bioavailability due to poor aqueous solubility and high metabolism, and is generally administered in liquid lipid vehicles. Solid-state formulations of CBD have been developed, but their ability to increase the oral bioavailability has not yet been proven *in vivo*. Various approaches are investigated to increase this bioavailability. This study aimed to demonstrate the enhancement of the oral bioavailability of oral solid dosage forms of amorphous CBD and lipid-based CBD formulation compared to crystalline CBD. Six piglets received the three formulations, in a cross-over design. CBD and 7 - COOH - CBD, a secondary metabolite used as an indicator of hepatic degradation, were analyzed in plasma. A 10.9-fold and 6.8-fold increase in oral bioavailability was observed for the amorphous and lipid formulations, respectively. However, the lipid-based formulation allowed reducing the inter-variability when administered to fasted animals. An entero-hepatic cycle was confirmed for amorphous formulations. Finally, this study showed that the expected protective effect of lipids against hepatic degradation of the lipid-based formulation did not occur, since the ratio CBD/metabolite was higher than that of the amorphous one.

Abbreviations

API = Active pharmaceutical ingredient

ASD = Amorphous solid dispersion

AUC_{last} = Area under the curve

BCS = Biopharmaceutics classification system

C_{max} = Maximum of concentration

CBC = Cannabichromene

CBD = Cannabidiol

CBG = Cannabigerol

DSC = Differential scanning calorimetry

EHC = Enterohepatic cycle

F = Absolute bioavailability

FaSSIF = Fasted state simulated intestinal fluid

GLP = Good laboratory pratices

ICH = International Committee of Harmonisation

MRM = Multiple reaction monitoring

MS = Mesoporous silica

T_{max} = Time at which C_{max} is reached

THC = 9Δ–tetrahydrocannabinol

UHPLC – MS/MS = Ultra-high pressure liquid chromatography – mass spectrometry (tandem)

UIR = Unit impulse response

Introduction

Endo- and phytocannabinoids systems are nowadays widely studied since they offer new possibilities of treatment. Actually, the most famous endocannabinoids, anandamide and 2-arachidonoylglycerol, have demonstrated bioactivity in pathways such as neurotransmission, analgesic properties, anxiolytics effects, memory information, neuroprotective effects or even fertility influence (Fonseca and Rebelo, 2022). Their receptors CB_1 and CB_2 interact also with phytomolecules known as phytocannabinoids. The most used phytocannabinoid is certainly 9Δ–tetrahydrocannabinol (THC) which is the most abundant in the *Cannabis* plant and is responsible of multiple effects including drug abuse. Besides THC, a plethora of molecules exists. Among them can be cited cannabidiol (CBD), cannabigerol (CBG) or cannabichromene (CBC). In addition to the capacity to interact with the endocannabinoids system, these molecules have antioxidant and anti-inflammatory properties thanks to their chemical structure. In particular, CBD is studied since it is also abundant in *Cannabis* plants and is devoid of any drug abuse risk unlike THC. This biphenolic compound is and was involved in many preclinical or clinical studies. Anxiety, schizophrenia, opioids use disorder or social phobia are examples of pathologies that could be treated at least partially with CBD (Bergamaschi et al., 2011; Heider et al., 2022). A retrospective study has highlighted positive clinical outcomes against moderate and severe symptoms of pain, anxiety and depression by given orally CBD-rich products such as CBD-rich cannabis oils that contain 20 – 25 mg of CBD/mL (Rapin et al., 2021). However, *Millar et al.* call for caution to all the virtues proposed by many authors since almost no phase III study has shown efficacy except against epilepsy (Millar et al., 2019). There are actually only two approved CBD-based medicines on the market. The first one is registered as an oily solution used to reduce seizures caused by Lennox-Gastaux and Dravet Syndrome (Epidiolex®, Greenwich Bioscience, Inc). The second one is a hydro-alcoholic solution that combines CBD with THC, it is used to reduce spasticity induced by multiple sclerosis (Sativex®).The oral bioavailability of CBD is estimated at 6% when solubilized in sesame oil (Millar et al., 2018; Perucca and Bialer, 2020) and could explain the poor phase III success. Absence of solid dosage form could be due to low aqueous solubility and low bioavailability. In addition, high inter- and intraindividual variability of CBD pharmacokinetic related to its low bioavailability is highlighted (Franco et al., 2020). This variability is a high drawback because plasmatic concentrations are hardly predictable and inevitably impacts clinical response, since preclinical and clinical studies do not personalize the doses used.

CBD is classified in the second category of the Biopharmaceutics Classification System (BCS) as its bioavailability is limited by the aqueous solubility and dissolution rate, which is

pH independent. In addition, this drug suffers from a high metabolism. Therefore, CBD needs challenged formulations to increase its bioavailability. Based on the physico-chemical properties of the drug, different strategies and technologies are employed to present the drug in its optimal form. As a lipophilic drug (LogP=6.3), CBD is often formulated with lipid excipient and/or taken with fat meal. As example and widely used as reference in many *in vitro* and *in vivo* studies, Epidiolex® contains refined sesame oil as a major solvent and ethanol as a co-solvent. The tested CBD formulations are also generally liquid and are presented as oil or self-emulsifying drug delivery systems (De Prá et al., 2021). This lipid strategy promotes the production of endogenous surfactant that will help the *in vivo* CBD solubilization. It potentiates in fact the lymphatic passage of CBD since it has been demonstrated that drugs with LogP above 5 have as their preferred absorption route transport via lymph (Trevaskis et al., 2008; Yáñez et al., 2011). This pathway is also the preferred route as fatty acids are present in intestinal cells. The formation of chylomicrons can thus transport lipophilic drugs directly into the systemic circulation via the lymph without passing through the liver. The liquid-lipid formulations have also some drawbacks considering the processability, the manufacturing, the stability and the patient compliance. Lipids are prone to oxidation reactions and the liquid state favors the instability of CBD. Some lipid components, such as sesame oil, have allergenic properties like many seed oils (Feeney et al., 2016). Besides the lipid strategy, many other formulations have been investigated. Although being a grease ball molecule, the use of the amorphous form of CBD prone to a high enhancement of CBD solubility and dissolution rate in vitro. As examples, amorphous solid dispersions (ASD) of CBD have been successfully developed as well as CBD-cyclodextrins complexes and mesoporous loaded silica (Jennotte et al., 2022; Koch et al., 2020). The mesoporous silica formulations allow obtaining free flowing powders of amorphous drugs thanks to high surface area of the inorganic carrier. If the adequate pore size and loading method are chosen, stable mesoporous materials with high degree of drug loading can be achieved (Koch et al., 2022). *In vivo* performances of mesoporous formulations have already been demonstrated for some BCS II drugs such as fenofibrate, celecoxib, asarone or itraconazole (Bukara et al., 2016; Mellaerts et al., 2008; Riikonen et al., 2015; Zhang et al., 2015).

The aims of this work were therefore to study for the first time the *per os* bioavailability of CBD in piglets in solid state within three different formulations: an amorphous formulation of CBD, a lipid-based CBD formulation and a crystalline CBD powder. Piglets were selected because they have a digestive tract similar to that of humans, including a gall bladder. Moreover, the hepatic metabolization of CBD within the formulation was evaluated by

following the concentration of the metabolite 7 – COOH – CBD, indicating potential protective effect from the first pass metabolism.

EXPERIMENTAL METHODS

Materials

CBD was purchased from THC Pharm (Germany); mesoporous silicas (MS) Syloid® XDP 3050 and Silsol® were kindly donated by W.R. Grace (Germany), Gelucire® 50/13 was kindly gifted by Gattefossé (France). FaSSIF powder was purchased from Biorelevant® (United Kingdom). Hydrochloric acid (HCl 37%) and natrium hydroxide were purchase from Merck® (Germany). CO2 (99.998%) was supplied by Air Liquide (Liège, Belgium). Deuterium-CBD, 7 – COOH – CBD and deuterium 7 – COOH – CBD were purchase from sigma Aldrich. Acetonitrile was HPLC grade, and methanol, water, ethyl acetate and n-hexane were UHPLC grade.

Production of CBD formulations

Amorphous formulation

Amorphous formulation used in this study has been developed and published by the current team (Koch et al., 2020). Further information such as the influence of the pore size could be found in the concerned publication. It consists of mesoporous silica Silsol (pore size: 6.6 nm) impregnated by CBD (60:40) in subcritical CO_2 conditions. A high-pressure cell (TOP Industrie, France) was filled with appropriate mass of Silsol MS and CBD. After closed, the cell was immerged in a water bath set at 50 °C and filled with CO_2 at the pressure of 60 bars, with a magnetic stirring set at 200 rpm. The system was finally depressurized after one hour. Final formulation was manually conditioned in 00 sized gelatin capsules without further formulation.

Lipid-based formulation

Lipid-based formulation was also developed by the current team (Koch et al., 2023). It consists of mixture of CBD and Gelucire® 50/13 incorporated in Syloid MS (pore size: 25 nm) in proportions 20:40:40 (CBD:Gelucire:XDP). CBD and Gelucire were melted in a water bath set at 70 °C. Once both components melted and homogeneously mixed, MS XDP was manually added in order to obtain a free flowing powder. Final formulation was manually conditioned in 00 sized gelatin capsules.

Crystalline formulation

Prior to conditioning in gelatin capsules, crystalline CBD powder was sieved through a 0.4 mm mesh sieve to avoid any agglomerate.

CBD formulations characterization:

Content and homogeneity

CBD content in each formulation has been determined in triplicate by using a HPLC-UV validated method (Koch et al., 2020). Zorbax® C18 300 SB analytical column with particles of 3,5µm (150 mm × 4.6 mm ID) was used with a mobile phase composed of a mixture of water and acetonitrile (38/62% v/v). The flow rate was set at 1.0 mL/min,the column temperature was kept constant at 30 °C and the detection wavelength was 240 nm. Formulations were dissolved in acetonitrile prior to sonication. Sonicated suspension were then filtered through 0.45 µm PTFE filter and analyzed.

Differential scanning calorimetry (DSC)

Approximatively 10 mg of powder was weighted, crimpled in an aluminum pan and then subjected to one heating ramp at a rate of 20 °K/min ranging from 25 °C to 100 °C to be within the range of CBD's melting point (68 °C). The equipment used for this study was a Mettler-Toledo® DSC 1 (Schwerzenbach, Swiss) controlled by the STARe System software.

Concentration at saturation of CBD in FaSSIF medium

The concentration at saturation of raw CBD in FaSSIF (fasted stated simulated intestinal fluid) has been determined by adding excess of CBD in media placed in a water bath set at 37 °C and a rotating speed of 100 rpm during 24 hours (triplicate). After this time, the suspension was filtered through PTFE 0.45 µm filter and diluted prior to HPLC analysis. FaSSIF medium was prepared as recommended by Biorelevant® (London, UK).

In vitro dissolution test

The *in vitro* dissolution performance of crystalline CBD and both amorphous and lipid-based formulations were tested and compared in simulated intestinal fluid FaSSIF (Biorelevant®, London, UK) media under sink conditions. FaSSIF has been chosen considering bioavailability studies performed with pig species (McCarthy et al., 2017; O'Shea et al., 2017; Perlstein et al., 2014). Dissolution tests were performed by using USPII paddle method (Sotax AT7, Suisse). For each formulation, equivalent of 10 mg of CBD in 00 sized gelatin

capsules were immersed in 500 mL of medium set at 37 °C. Dissolution tests have been done during 4 hours (withdrawals at 5, 15, 30, 45, 60, 90, 120, 180 and 240 minutes) in triplicate with a paddle speed set at 100 rpm. Every withdrawal was filtered through 0.45 µm PTFE filter prior to HPLC-UV dosage. Means and standard deviations of *in vitro* data were calculated using GraphPad Prism 5.0 software.

In vivo **study**

In vivo study was performed within PigForLife® (CER Group Facilities) GLP structure (Marloie, Belgium). The study protocols were approved by the Local Ethical Committee before the start of the study and complied with the Belgian regulation as published in «Royal Decree of May 29, 2013 on the protection of experimental animals, A.G.W. 30.11.2017».

Per os study was conducted by given orally an equivalent of 2.5 mg CBD/kg in fasted sate. This dosage was selected based on Epidiolex® posology and dosage form volume. The amorphous and lipid-based formulations were compared to the crystalline CBD. All formulation were sealed manually in gelatin 00 sized capsules without any additional excipient and administered by force-feeding system. Six Large White piglets (male and female, between 8 and 20 kg) born on site and in a healthy condition were used. Due to the exploratory nature of the study, a number of six was chosen arbitrary. The animals were housed together in pens of about 40m² and enriched environment was provided. Natural temperature and light cycle were used in a straw litter. During storage, animals were *ad libitum* fed with pellet food balanced without any curative or preventive veterinary medicinal products or veterinary additives. Tap water was available *ad libitum* during the entire study period, including accommodation and sampling days. Piglets were fasted overnight (food but not water) before giving the formulations and were then fed 6h and 12h post-dose to avoid hypoglycemia. All piglets were clinically examined before the study and before sampling. Each piglet received the three formulations in a randomized order with a wash-out period of one week (Table 1).

Blood sampling (5 mL) were done in EDTA tube at time zero, 1h, 2h, 2.5h, 3h, 3.5h, 4h, 4.5h, 5h, 5.5h, 6h, 7h, 8h, 12h and 24h. The samples were stored at 4°C and then centrifuged at 2500g for 10 minutes. Plasma samples were separated from the figurative elements of blood and were frozen at -80°C until their dosage.

Table 1 - Randomization of the *per os* study

Piglet	Week 1	Week 2	Week 3	Week 4
P1	Amorphous	Lipid-based	x	Crystalline
P2	Crystalline	Amorphous	Lipid-based	x
P3	x	Crystalline	Amorphous	Lipid-based
P4	Lipid-based	x	Crystalline	Amorphous
P5	Amorphous	Lipid-based	x	Crystalline
P6	Crystalline	Amorphous	Lipid-based	x

Bioanalysis

CBD and 7 – COOH – CBD were simultaneously quantified by a validated UHPLC – MS/MS method based on ICH M10 guidelines (Koch et al., 2024). Briefly, 500µL of plasma samples were fortified with 20 µL of internal standards solution (250 ng/mL of cannabidiol-d_3 and 2000 ng/mL of 7 – COOH – CBD –d_3), 100 µL of 10% anhydrous glacial acetic acid and analytes were extracted with 5mL of n-hexane/ethyl acetate (9/1 v/v) by stirring and centrifugation processes. The supernatant was transferred into vials prior to drying. Once dried, 100 µL of methanol/water (50/50) was added to dissolve the residue and transferred to Eppendorf vials prior to centrifugation and UHPLC vial filling. Samples were analyzed with a Water Xevo™ TQ-S mass spectrometer interfaced with a Waters Acquity® UPLC I-Class inlet system. An Acquity® UPLC BEH C18 (1.7µm (2.1 x 50 mm)) column set at 45°C was used as stationary phase and the mobile phase consisted in a mixture of ammonium bicarbonate buffer (pH10) and methanol in a gradient mode, with a flow rate of 0.45mL/min. Injection volume was 1µL for a run time of 6 minutes. Positive electrospray ionization mode was used and two MRM transitions (m/z) were used for identification and quantification of CBD and 7 – COOH – CBD. Calibration curves and QC were daily prepared and data acquisition was achieved using MassLynx Version 4.2 software and TargetLynx Version 4.2 software.

Pharmacokinetic analysis

Concentrations versus time *in vivo* data and pharmacokinetic parameters C_{max}, T_{max} and AUC_{last} were analyzed by a non-compartmental pharmacokinetic analysis using Phoenix™ WinNonlin 8.4 (Certara, Princeton, USA). Linear trapezoidal linear interpolation calculation method was applied for AUC_{last}, C_{max} correspond to the highest concentration observed and T_{max} is the time requested to achieve C_{max}. Deconvolution process has been performed by

using unit impulse response (UIR) from intravenous data in piglet provided by the current team and published elsewhere in order to construct the input rate and fraction absorption graphs (Koch et al., 2024). All measured concentrations (*C*) were normalized with the administered dose. If the concentration at T_0 (C_0) differed from 0 ng/mL, correction was made by using the following formula (Eq.1):

$$Corrected\ concentration\ (\ If\ C0 \neq 0\ ng/mL) = C - (C0)^{t*0.34} \qquad (Eq.1)$$

Where C_0 is the concentration observed at time 0h, t the time of sampling, 0.34 is the overall terminal elimination obtained after intravenous administration (Koch et al., 2024). Phoenix™ WinNonlin 8.4 software was used to calculate the means and standard deviations of *in vivo* data.

Results and discussion

CBD content and physical state

Results on drug content analysis by HPLC revealed that CBD content in all used formulations of this study was between 95 and 105%, suggesting that formulation process induced no significant drug degradation or loss. Additionally, amorphization of CBD within the amorphous and lipid-based formulations was confirmed by DSC prior to *in vitro* dissolution and *in vivo* study as shown on Figure 1. The formulations were therefore stable for all the duration of the current *in vitro* and *in vivo* experiments.

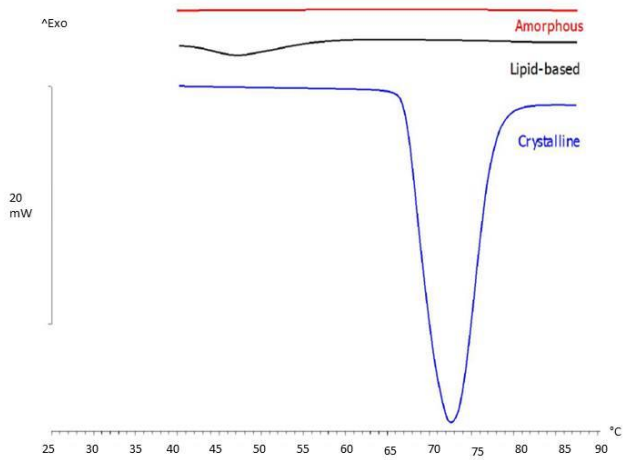


Figure 1 – Thermograms of amorphous and lipid-based formulations, compared to the crystalline CBD

Both lipid-based and amorphous formulations presented a non-crystalline state of CBD. Amorphous formulation is a purely non organized state of CBD stabilized inside mesoporous network while the lipid-based formulation is a solid solution of CBD within the lipid material. The release of CBD from the amorphous formulation is inherent of its interaction with the silica surface. On the other side, the lipid-based formulation presents CBD in a fine interaction with lipids contained in the formulation. The release of CBD outside the silica is therefore dependent on a preliminary step of solubilizing the CBD-Gelucire® 50/13 mixture inside the mesopores, which must then exit the silica pores by diffusion.

In vitro **dissolution test**

FaSSIF medium simulates the fasted intestinal fluid with a pH of 6.5 and contained 3 mM of taurocholate and 0.75 mM of phospholipids, namely lecithin. This media mimics the intestinal fluids in terms of pH, isotonicity and surfactant characteristics, under fasted conditions (Henze et al., 2019). The concentration at saturation of crystalline CBD in FaSSIF medium has been determined at 62.17 ± 2.70 µg/mL. As the actual water solubility of CBD is given in the literature at 0.39 µg/mL (Grifoni et al., 2022), this result shows the importance of the presence of surfactants and/or phospholipids to increase the CBD solubility. Since CBD is a BCS II molecule, its bioavailability is theoretically limited by the solubilization. Sink conditions were thus chosen since it is expected that all drug dissolved will readily be absorbed. The dissolution should therefore be little or not influenced by what has already been dissolved. Equivalent of 10 mg were tested. The dissolution rate of the crystalline CBD was very slow and only 6.72 ± 0.86% of CBD was dissolved after four hours. The release profile of the crystalline form was not studied further, as its kinetic was too slow compared with the other two formulations. With the two other formulations, dissolution tests showed a rapid but no immediate release of CBD since 80% are dissolved after 60 min and 90 min for the amorphous and the lipid-based formulation respectively (Figure 2). This could be explained by the more complex solubilization process of the lipid-based formulation discussed further. Formulating CBD as pure amorphous state or in mixture with lipid allow a complete release *in vitro* of the drug after 180 min.

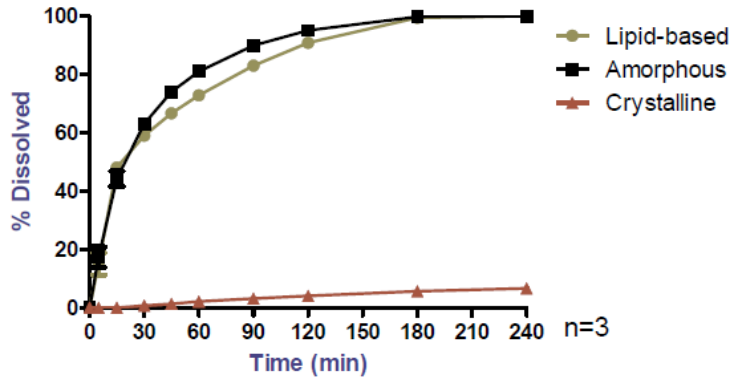


Figure 2 – *In vitro* dissolution tests of crystalline, amorphous and lipid-based formulations in FaSSIF medium (n=3; ± SD)

The dissolution data were fitted to Weibull equation model (Eq. 2; Figure 3).

$$Finf * \left(1 - e^{\left(\frac{t}{MDT}\right)}\right)^{B} = \%\, dissolved \qquad \text{(Eq.2)}$$

Where F_{inf} is the maximum of dissolution (i.e. 100% if dissolution is complete), t the time, MDT the mean dissolution time and b the slope factor. Amorphous and lipid-based formulations have a Weibull kinetics release profile under sink conditions in FaSSIF media as evidenced by correlation coefficients of 0.999 and 0.997 respectively.

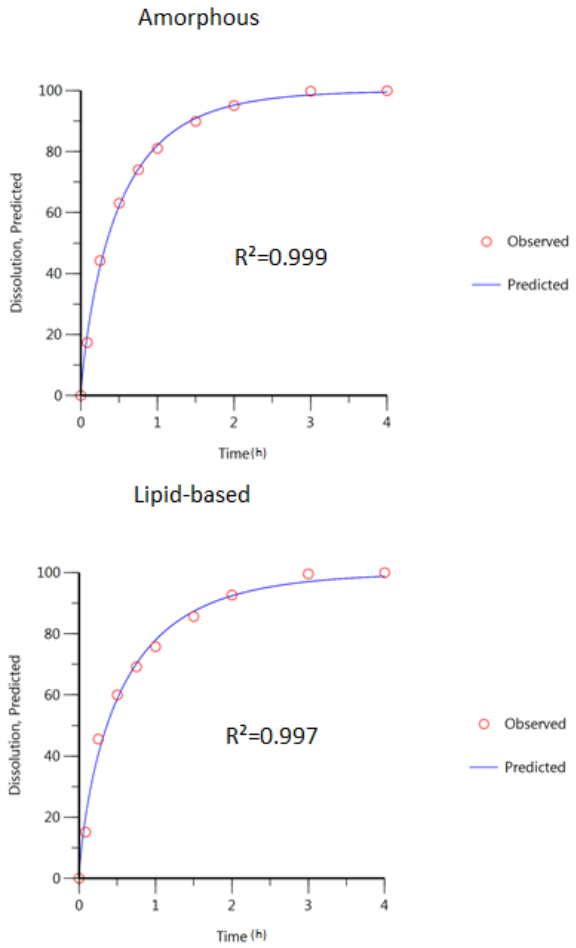


Figure 3 – Observed and Weibull based predicted values plots

The b exponent, determined at 0.82 and 0.77 for the amorphous and lipid-based formulation respectively, characterizes the mechanism of exponential drug release. When the b value is situated between 0.75 and 1, the release mechanisms are a combination of diffusion and another effect (Papadopoulou et al., 2006). As example, Corsaro et al. described a combination of Fickian diffusion and swelling controlled transport (Corsaro et al., 2021). If swelling effect could occur with the lipid-based formulation, the amorphous one is not susceptible to it. The fit considering the Korsmeyer-Peppas equation (Eq.3) give n-values below 0.43 for both formulation, which also indicates a predominant Fickian diffusion (Table 2) (Tomic et al., 2018).

$$\% \, dissolved = K * t^n = \frac{Mt}{M\infty} \qquad (Eq. 3)$$

Where K is the release rate constant, n is the release exponent, Mt/M∞ is the drug release at time t.

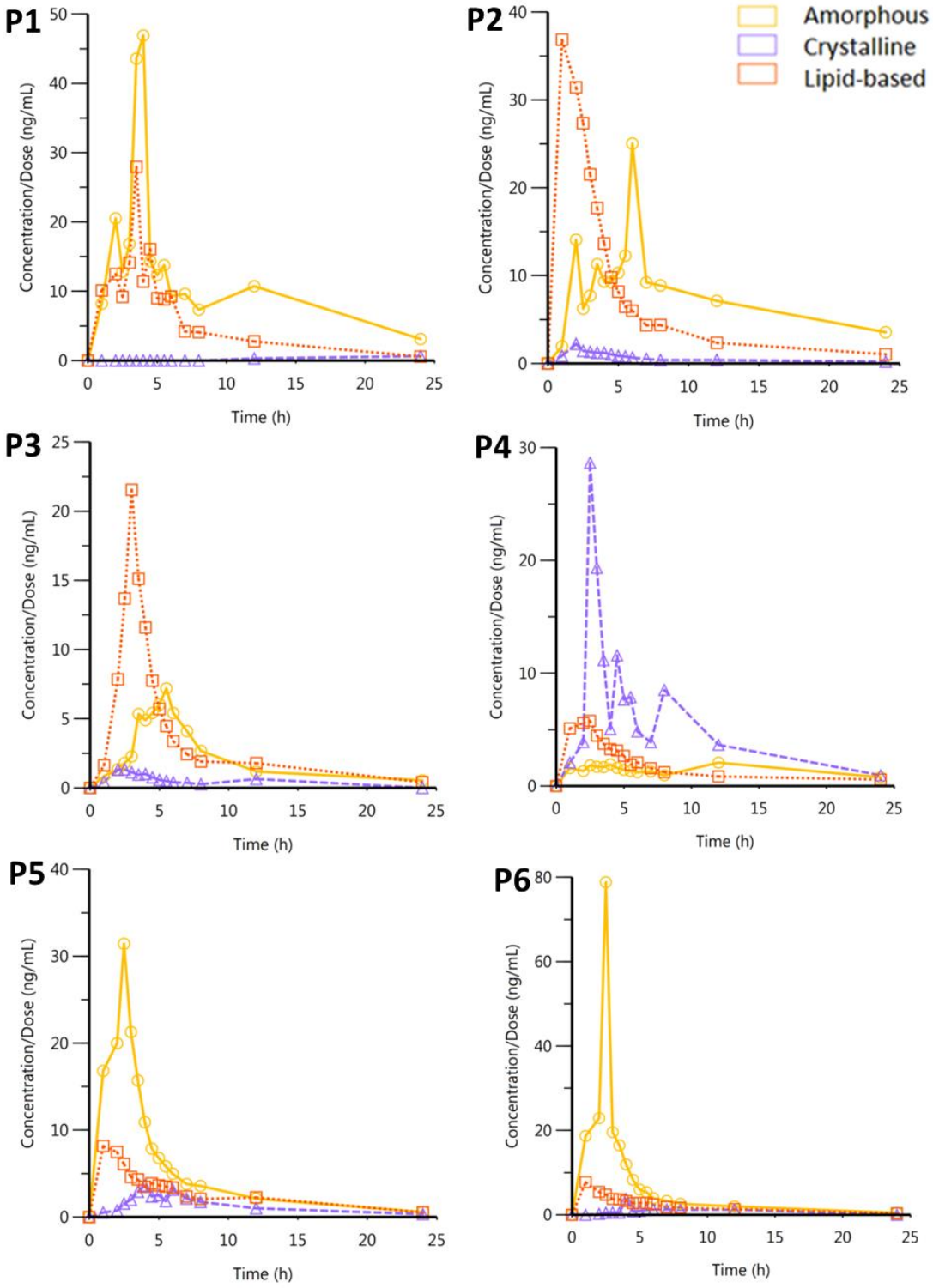
While both formulations initially showed a CBD release related to the gradient concentration, the amorphous formulation sees its release slow down over time probably due to the need for fluids to go deeper into the pores of mesoporous silica, and the lipid-based formulation sees the addition to the two precedent release mechanism of a lipid digestion process.

Table 2 - Korsmeyer - Peppas equation parameters

Fitting	K	n	r^2
Lipid-based	19.19	0.32	0.977
Amorphous	21.27	0.30	0.971

In vivo **study**

All animals were daily checked during the study and evident signs of toxicity, general appearance, viability, mobility and stools were examined and no abnormality was detected. The wash-out period used in this study (one week) was adequate since almost all T_0 were quantified at 0 ng/mL, except for two single time points. Among the six piglets, one non-concordant animal was detected. Indeed, the bioavailability of crystalline CBD in one specific piglet (P4) was much higher than the two formulations and that were completely different from the results obtained with the five others piglets (Figure 4). P4 was in fact the one concerned by the presence of the two time point concentrations at T_0 which differed from 0 ng/mL. To take into account this afterglow, the concerned plasmatic concentration values were corrected as detailed above (Eq. 1). For P1, P2, P3, P5 and P6, amorphous and lipid-based formulations allow a significant enhancement of bioavailability of CBD in comparison with the crystalline CBD. Moreover, the individual concentration *vs* time curves show a high variability between the animals. It is observed that the amorphous and lipid-based curves never show similar exponential phases from T_0 to T_{max} for a same piglet. Pharmacokinetic parameters T_{max}, C_{max}, and AUC_{last} calculated from the data using the six piglets showed higher variability for the amorphous formulation in comparison with the lipid-based formulation (Table S1, Supplementary materials). This may be due to the fact that the formulations require different digestive process and metabolism pathway to *in fine* allow the CBD release and absorption. The genetic variability provides piglets with different metabolic and digestive capacity (Noblet et al., 2013). As access to water was not controlled, some piglets could have drink more and have accelerated transit with for example less contact with gastric medium or diluted gastric fluid with higher pH. These factors may have influenced the CBD absorption from the two optimized formulations and from crystalline materials, leading partially to the observed intervariability.



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346 **Figure 4 – Individual time vs concentration curves of CBD (2.5 mg/kg) for the three formulations (Amorphous, lipid-based**
347 **and crystalline). Please note that the scale is different in function of the piglet.**

As the fourth piglet (P4) showed a completely different behavior and showed a high AUC and C_{max} for the crystalline form of CBD, it was decided to exclude this piglet from the main analysis since it modified considerably the results and biased the bioavailability assessment.

Attention has to be made on the fact that the results directly depend on the variability. In particular, T_{max} values are highly influenced by the presence of at least two peaks for the amorphous formulation while the crystalline and lipid-based formulations have mostly one single peak. T_{max} is indeed associated with the C_{max} and not always with the first peak. This problematic has been widely reviewed and authors discussed the fact that choosing to work with mean or individual curves may considerably influence the interpretation of the results (Cardot and Davit, 2012). In this work, we choose to provide first individual-curves-related values and secondly to use the mean curves. While the AUC_{last} of crystalline CBD reached 13.25 ± 8.82 ng*h*mL^{-1}, amorphous and lipid-based formulations reached respectively 144.47 ± 72.55 and 89.97 ± 46.00 ng*h*mL^{-1} (Table 3). This is an increase in relative bioavailability of 10.90 and 6.79 fold for the amorphous and lipid-based formulation. T_{max} was reached after about 4.1 ± 1.64 hours for the amorphous formulation (C_{max} = 37.87 ± 26.95 ng/mL), 1.9 ± 1.24 hours lipid-based formulation (C_{max} = 20.44 ± 12.65 ng/mL) and 7.3 ± 9.38 hours for crystalline CBD (C_{max} = 2.31 ± 1.37 ng/mL). For amorphous formulation, a first peak is observed, close to that observed for lipid-based formulation. However, this peak is not always correlated with the highest concentration and is therefore not calculated as a T_{max} but must be taken into account when studying the speed at which CBD appears in the blood. Indeed, the first peak provided by the amorphous formulation appears at similar time than T_{max} of lipid-based formulation. This could be correlated with the similar *in vitro* dissolution rate of both formulations.

Table 3 – Mean (±SD) of pharmacokinetic parameters obtained from individual curves (CBD dose 2.5 mg/kg; n=5)

	Amorphous	Lipid-based	Crystalline
AUC_{last} (ng*h*mL^{-1})	144.47 ± 72.55	89.97 ± 46.00	13.25 ± 8.82
C_{max} (ng/mL)	37.87 ± 26.95	20.44 ± 12.65	2.31 ± 1.37
T_{max} (h)	4.1 ± 1.6	1.9 ± 1.2	7.3 ± 9.4

Amorphous formulation presented virtually a high quantity of isolate molecules of CBD that are directly prone to be solubilized and absorbed. Indeed, DSC results suggest a complete amorphization of the material and a monolayer loading is approached. Once absorbed, the amorphous CBD-loaded silica particles release the CBD rapidly and with high intensity because no other release mechanism occurs than Fickian diffusion. Besides, the lipid-based formulation must follow several steps before presenting the CBD in solubilized state in the intestinal tract. These differences may lead to the higher AUC_{last} and higher C_{max} observed

with the amorphous formulation in comparison with the lipid-based formulation. Interestingly, these results are in accordance with the work of *Bukara et al.* who compared the enhancement of fenofibrate bioavailability from an ordered mesoporous formulation and from the commercial lipid product Lipanthyl® in humans. Equally to the results presented in our study, their amorphous formulation provided a higher C_{max} normalized per dose and higher AUC_{last} in comparison to the lipid-based formulation (Bukara et al., 2016). Fenofibrate is actually a grease ball BCS II molecule used to treat hyperlipidemia with similar LogP and melting point as CBD. Our hypothesis is that the absorption of CBD from the amorphous formulation depends on the quantity of digestive fluid (that contains endogenous surfactants) present at the moment of absorption, whereas this is less the case for the lipid-based formulation. Given that some piglets are likely to have high capacity to absorb CBD from the amorphous formulation into the bloodstream, some C_{max} are high and increase the mean value. The amorphous formulation therefore requires the presence of physiological elements enabling absorption. The lipid formulation seems more complicated to absorb, but more homogeneous between piglets, which is explained by the presence of self-emulsifying lipids in the formulation, enabling equivalent digestion in all piglets, whatever their digestive capacity.

Deconvolution process by using UIR from a two compartment model provided by data obtained with an intravenous pharmacokinetic study in piglet (Koch et al., 2024) allowed constructing the CBD absorption profile from the three tested formulations. Absolute bioavailability (F) obtained from the five piglets is shown on Figure 5. Although the amorphous formulation achieved the highest absorption, it also suffered from high variability, unlike the lipid-based formulation. As the formulations were given to fasted piglets, the self-emulsification properties of the Gelucire® 50/13 reduced the inter-variability since every piglet started equally the study as they received an adequate and identical quantity of surfactant via the formulation. It is clear that both formulations allowed a high enhancement of CBD absorption in comparison with the pure and crystalline form thanks to an increase solubilization capacity.

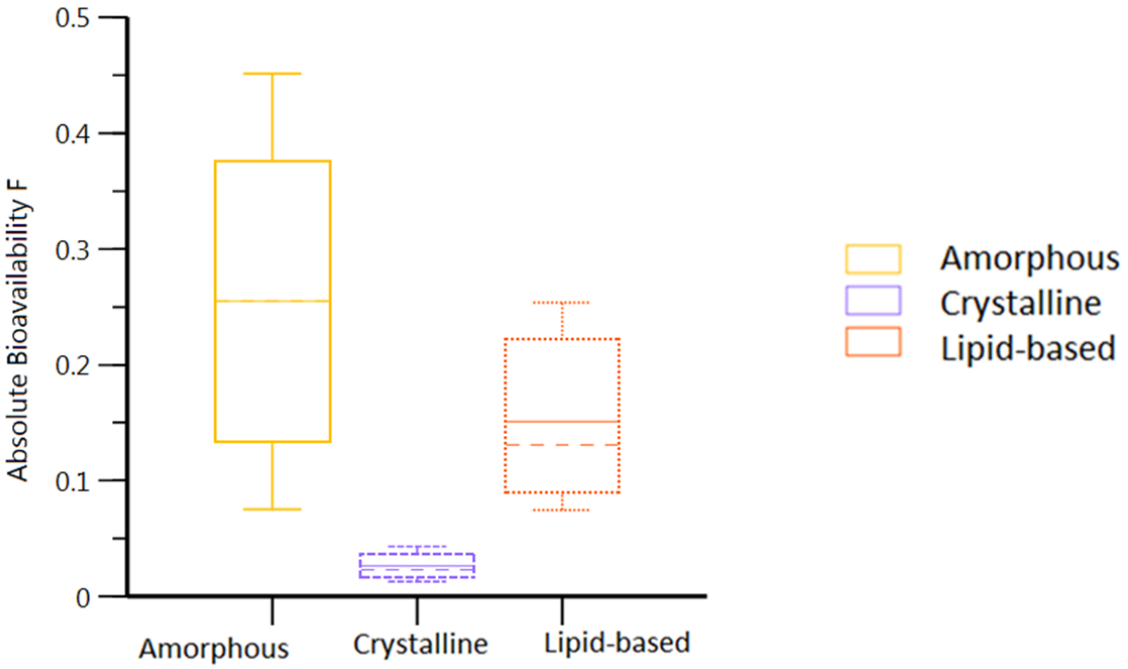

Figure 5 – Bioavailability of CBD depending on the formulation (n=5)

The mean curves showed an apparent enterohepatic cycle (EHC), mainly for amorphous formulation, as shown on Fig 6. Indeed, the multiple peaks observed with the amorphous formulation reflect the individual curves and be possible EHC. For the lipid-based formulation, the observed plateau is less reflecting individual curves.

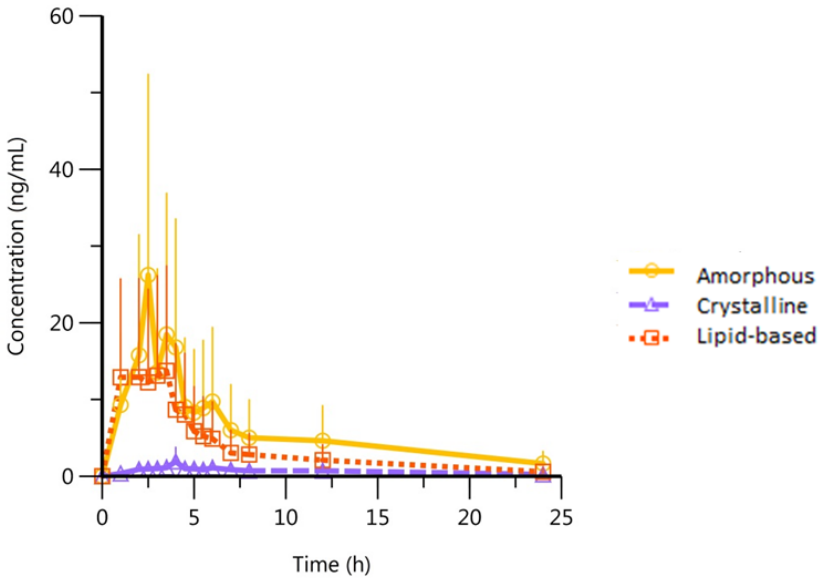

Figure 6 - Mean curves (± SD) of CBD concentration following administration of 2.5 mg/kg (n=5)

As a reminder, EHC occurs when either a glucuroconjugated metabolite formed in the liver - or untransformed drug - passes through the gallbladder and is released in the intestinal tract with bile. In contact with digestive enzymes, the glucuroconjugate may revert to its original form and be reabsorbed; resulting in a second, or even third, peak often linked with time of meals. This effect leads to a longer contact of the body with the drug and increases thus the AUC_{last}. The question arises as to whether the apparent EHC observed on the mean curves is due to a real reabsorption of product release by the bile or whether this EHC form-like is due to a large variability in terms of T_{max} depending on the individual. Again, the problem of using the individual or the mean curves is highlighted. If the figure 4 showed two peaks when amorphous formulation was administrated, the lipid-based formulation showed two populations of monopeaks characterized by different T_{max}. By merging all the data, two peaks or a plateau appear for the mean curve of the lipid-based and the amorphous formulation, but are probably of different origin. Figure 7 shows the input rate of CBD absorption in function of the animal and of the formulation (amorphous or lipid-based). It is clear that the amorphous formulation presents a profile with at least two early peaks of absorption before 5 h, followed by a third smaller one, for all animal. The two first peaks occur therefore independently of any meals administration as the animals were still fasted until the time point 6h. The third peak is less surprising, since it is linked to the first meal following administration of the formulation. As the dissolution process from the amorphous formulation is not characterized with successive release mechanisms, the successive phases of *in vivo* absorption may indicates reabsorption and thus an EHC.

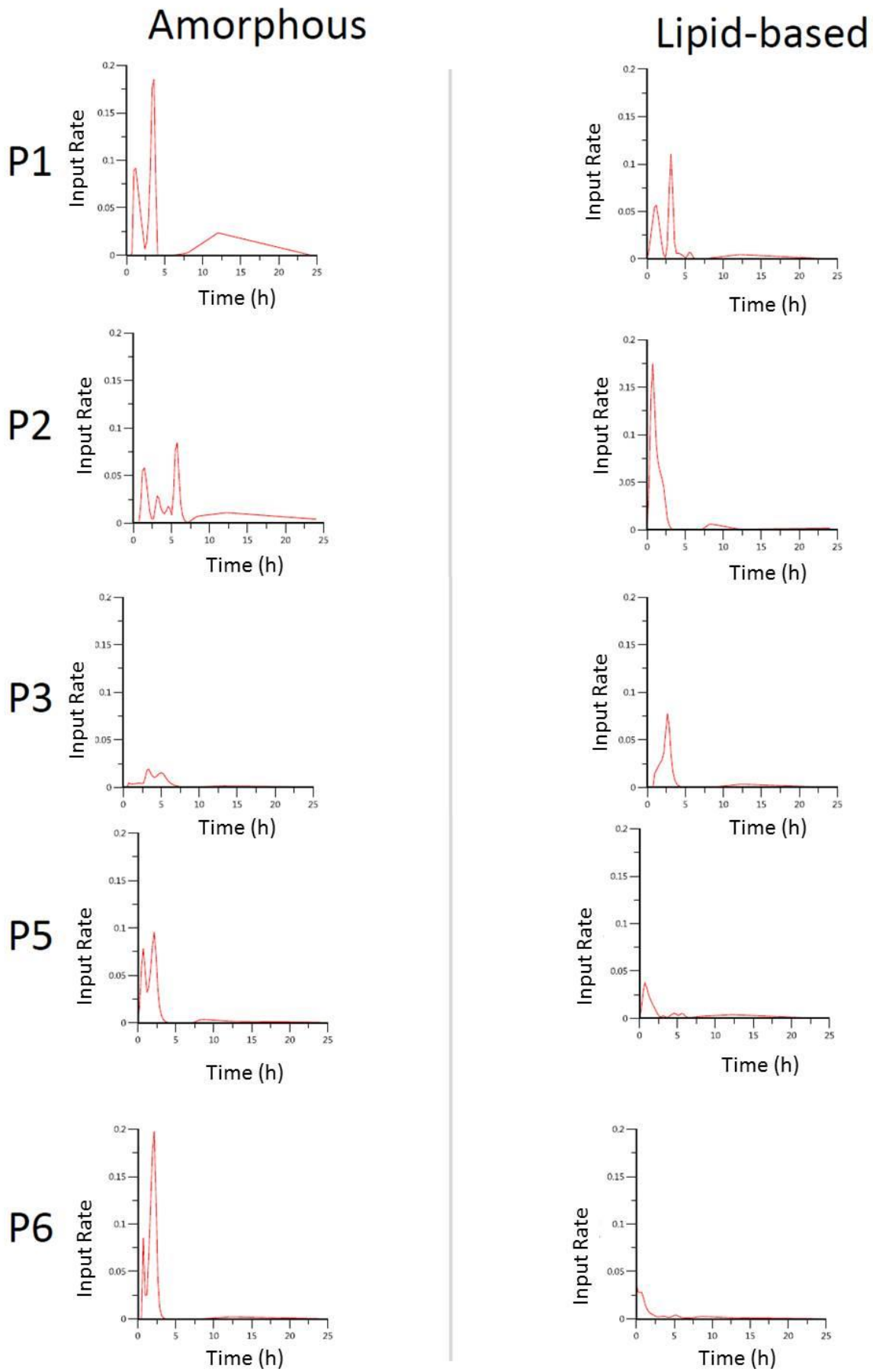

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Figure 7 – Input rate of CBD absorption depending on animal and formulation

Considering the lipid-based formulation, if the later peak (corresponding to the third of amorphous form and linked to the 6h post-dose meal) is also present among all animals, the two first peaks are not always present. EHC may not systematically occur with the lipid-based formulation and the apparent EHC would be rather due to the superimposition of early population (T_{max} of 1 hour for P2, P5 and P6) and late population (T_{max} of 3 – 3.5 hours for P1 and P3)

As 7 – COOH – CBD was simultaneously quantified, the hepatic first pass degradation and metabolization could be estimated. Figure 8 presents the mean profiles of 7 – COOH – CBD, product of CBD metabolization, depending on the CBD formulation. This metabolization occurs to provide more hydrophilic compound in order to accelerate the elimination of the drug from the body. This secondary metabolite is inactive and follows the oxidation of the first and active metabolite 7 – OH – CBD. These oxidations are described to mainly occur in the liver by cytochromes enzymes and may thus partially represent the first-pass. However, intravenous data showed that this first-pass is not on itself responsible of the early production of oxidized metabolite. The formation of the carboxylate metabolite is thus due by a combination of first-pass and systemic metabolization. Some lipid formulations are used to overcome the first-pass metabolization. Authors have shown for example that the administration of fatty acid (especially C16 and C18) in combination with lipophilic drugs, which are prone to the first-pass effect, favor the formation of chylomicrons and the lymphatic absorption (Franco et al., 2020). This lymphatic absorption allows to by-pass the liver first-pass and so increases the bioavailability.

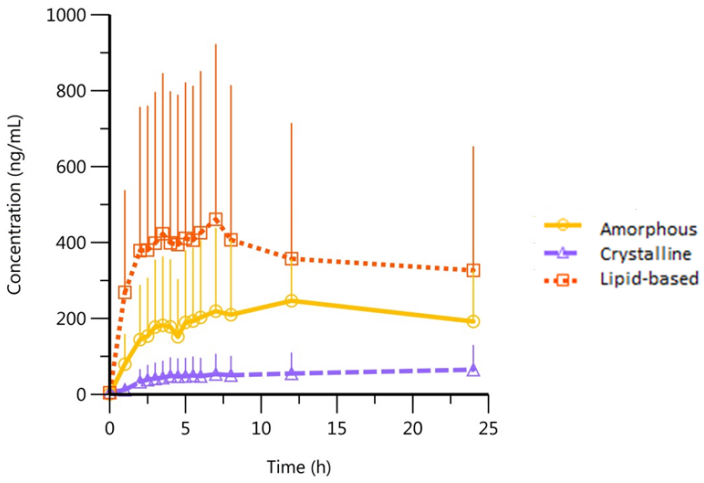

Figure 8 - 7 - COOH - CBD mean curves (± SD) after administration of an equivalent of 2.5 mg of CBD/kg (n=5)

Actually, the results were not expected since it was postulated that Gelucire protected CBD to liver degradation by favoring the lymphatic transport. However, the AUC_{last} ratio metabolite/CBD is much higher with the lipid-based formulation (95.33) than with the amorphous formulation (33.37). The ratio obtained with the lipid formulation is quite similar than the pure and crystalline CBD (93.87). The phenomenon explaining why the amorphous form of CBD provided higher bioavailability and less hepatic degradation remains unclear and precise investigations have to be conducted. It seems however intuitive that the mesoporous silica amorphous loaded CBD lead to a tank that promotes supersaturation of CBD within intestinal fluids. This supersaturated concentration increases the gradient and accelerates the absorption rate. The hepatic enzymes are therefore more susceptible to be saturated, promoting the passage of non-metabolized CBD in the bloodstream, translated by a high AUC and low CBD/metabolite ratio and could explain higher variability as linked with hepatic status and supersatured state. In addition, it has been shown that CBD suffer from a rapid systemic degradation when intravenously administrated. Since CBD metabolism is a combination of hepatic and systemic degradation, the presence of lipids in the lipid-based formulation involved in the lymphatic passage has only a limited impact on protection against degradation. All of these differences may lead to the higher CBD bioavailability observed with the amorphous formulation in comparison with the lipid-based formulation. Thanks to an enterohepatic cycle and a reduced metabolization, the amorphous formulation allows increasing the AUC_{last} of CBD, whose absorption seems to however depend on the presence of digestive fluids and may lead to high variability. The lipid-based formulation, on the other hand, offers the advantage of more reproducible bioavailability and less dependence on digestive capacity, although it is smaller due to the absence of systematic enterohepatic cycle and greater metabolism. While both the amorphous and lipid-based strategies are interesting, the lipid-based formulation could be of greatest interest from a marketing perspective, due to its definite increase in bioavailability, but also its lower variability when administered to fasting individuals. This kind of formulation therefore reduces the food effects (O'Shea et al., 2015), enabling better control of plasma concentrations and hence therapeutic effects. What's more, the lipid-based formulation is very simple to produce and requires no special equipment, making it easy to scale-up.

Conclusion

A CBD amorphous and CBD lipid-based formulations were tested and compared with crystalline CBD in *in vitro* and *in vivo* conditions. Both optimized formulations allowed a complete release of CBD within a maximum of 180 minutes in FaSSIF medium as the lipid-based one slower the release due to more complex solubilization process. Both formulations

increased the bioavailability of CBD by 10.9-fold and 6.8-fold for the amorphous and lipid formulations, respectively. A high inter piglet variability has been highlighted as well as a potential enterohepatic cycle mainly for the amorphous formulation. The simultaneous quantification of the metabolite 7 - COOH - CBD shows a higher metabolization from the lipid-based formulation than from the amorphous one, demonstrating that the lipid strategy was not advantageous to avoid the first pass effect. Besides, it has been shown that the lipid-based formulation allows reducing the absorption variability.

Supplementary materials

Table S1 - Mean of pharmacokinetic parameters (± SD) obtained from individual curves, including P4 (CBD dose 2.5 mg/kg; n=6)

	Amorphous	Lipid-based	Crystalline
AUC_{last} (ng*h*mL^{-1})	126.07 ± 79.00	81.34 ± 46.25	30.09 ± 42.02
C_{max} (ng/mL)	31.89 ± 28.23	17.99 ± 12.80	6.71 ± 10.83
T_{max} (h)	5.4 ± 3.54	2.0 ± 1.14	6.5 ± 8.61

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References

Bergamaschi, M.M., Queiroz, R.H.C., Chagas, M.H.N., De Oliveira, D.C.G., De Martinis, B.S., Kapczinski, F., Quevedo, J., Roesler, R., Schröder, N., Nardi, A.E., Martín-Santos, R., Hallak, J.E.C., Zuardi, A.W., Crippa, J.A.S., 2011. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-nave social phobia patients. Neuropsychopharmacology 36, 1219–1226. https://doi.org/10.1038/npp.2011.6

Bukara, K., Schueller, L., Rosier, J., Martens, M.A., Daems, T., Verheyden, L., Eelen, S., Van Speybroeck, M., Libanati, C., Martens, J.A., Van Den Mooter, G., Frérart, F.,

Jolling, K., De Gieter, M., Bugarski, B., Kiekens, F., 2016. Ordered mesoporous silica to enhance the bioavailability of poorly water-soluble drugs: Proof of concept in man. Eur. J. Pharm. Biopharm. 108, 220–225. https://doi.org/10.1016/j.ejpb.2016.08.020

Cardot, J.M., Davit, B.M., 2012. In vitro-in vivo correlations: Tricks and traps. AAPS J. 14, 491–499. https://doi.org/10.1208/s12248-012-9359-0

Corsaro, C., Neri, G., Mezzasalma, A.M., Fazio, E., 2021. Weibull modeling of controlled drug release from Ag-PMA nanosystems. Polymers (Basel). 13. https://doi.org/10.3390/polym13172897

De Prá, M.A.A., Vardanega, R., Loss, C.G., 2021. Lipid-based formulations to increase cannabidiol bioavailability: In vitro digestion tests, pre-clinical assessment and clinical trial. Int. J. Pharm. 609. https://doi.org/10.1016/j.ijpharm.2021.121159

Feeney, O.M., Crum, M.F., McEvoy, C.L., Trevaskis, N.L., Williams, H.D., Pouton, C.W., Charman, W.N., Bergström, C.A.S., Porter, C.J.H., 2016. 50 years of oral lipid-based formulations: Provenance, progress and future perspectives. Adv. Drug Deliv. Rev. 101, 167–194. https://doi.org/10.1016/j.addr.2016.04.007

Fonseca, B.M., Rebelo, I., 2022. Cannabis and Cannabinoids in Reproduction and Fertility: Where We Stand. Reprod. Sci. 29, 2429–2439. https://doi.org/10.1007/s43032-021-00588-1

Franco, V., Gershkovich, P., Perucca, E., Bialer, M., 2020. The Interplay Between Liver First-Pass Effect and Lymphatic Absorption of Cannabidiol and Its Implications for Cannabidiol Oral Formulations. Clin. Pharmacokinet. 59, 1493–1500. https://doi.org/10.1007/s40262-020-00931-w

Grifoni, L., Vanti, G., Donato, R., Sacco, C., Bilia, A.R., 2022. Promising Nanocarriers to Enhance Solubility and Bioavailability of Cannabidiol for a Plethora of Therapeutic Opportunities. Molecules 27. https://doi.org/10.3390/molecules27186070

Heider, C.G., Itenberg, S.A., Rao, J., Ma, H., Wu, X., 2022. Mechanisms of Cannabidiol (CBD) in Cancer Treatment: A Review. Biology (Basel). 11. https://doi.org/10.3390/biology11060817

Henze, L.J., Koehl, N.J., O'Shea, J.P., Kostewicz, E.S., Holm, R., Griffin, B.T., 2019. The pig as a preclinical model for predicting oral bioavailability and in vivo performance of pharmaceutical oral dosage forms: a PEARRL review. J. Pharm. Pharmacol. 71, 581–

602. https://doi.org/10.1111/jphp.12912

Jennotte, O., Koch, N., Lechanteur, A., Evrard, B., 2022. Development of amorphous solid dispersions of cannabidiol: Influence of the carrier, the hot-melt extrusion parameters and the use of a crystallization inhibitor. J. Drug Deliv. Sci. Technol. 71, 103372. https://doi.org/10.1016/j.jddst.2022.103372

Koch, N., Jennotte O., Ziemons E., Boussard G., Lechanteur A., E.B., 2022. Influence of API physico-chemical properties on amorphization capacity of several mesoporous silica loading methods. Int. J. Pharm. 613, 121372. https://doi.org/https://doi.org/10.1016/j.ijpharm.2021.121372

Koch, N., Jennotte, O., Gasparrini, Y., Vandenbroucke, F., Lechanteur, A., Evrard, B., 2020. Cannabidiol aqueous solubility enhancement: Comparison of three amorphous formulations strategies using different type of polymers. Int. J. Pharm. 589. https://doi.org/10.1016/j.ijpharm.2020.119812

Koch, N., Jennotte, O., Lechanteur, A., Deville, M., Charlier, C., Cardot, J., Chiap, P., Evrard, B., 2024. An Intravenous Pharmacokinetic Study of Cannabidiol Solutions in Piglets through the Application of a Validated Ultra-High-Pressure Liquid Chromatography Coupled to Tandem Mass Spectrometry Method for the Simultaneous Quantification of CBD and Its Carboxylated Metabolite in Plasma. Pharmaceutics 16(1). https://doi.org/https://doi.org/10.3390/pharmaceutics16010140

Koch, N., Jennotte, O., Toussaint, C., Lechanteur, A., Evrard, B., 2023. Production challenges of tablets containing lipid excipients: Case study using cannabidiol as drug model. Int. J. Pharm. 633. https://doi.org/10.1016/j.ijpharm.2023.122639

McCarthy, C.A., Faisal, W., O'Shea, J.P., Murphy, C., Ahern, R.J., Ryan, K.B., Griffin, B.T., Crean, A.M., 2017. In vitro dissolution models for the prediction of in vivo performance of an oral mesoporous silica formulation. J. Control. Release 250, 86–95. https://doi.org/10.1016/j.jconrel.2016.12.043

Mellaerts, R., Mols, R., Jammaer, J.A.G., Aerts, C.A., Annaert, P., Van Humbeeck, J., Van den Mooter, G., Augustijns, P., Martens, J.A., 2008. Increasing the oral bioavailability of the poorly water soluble drug itraconazole with ordered mesoporous silica. Eur. J. Pharm. Biopharm. 69, 223–230. https://doi.org/10.1016/j.ejpb.2007.11.006

Millar, S.A., Stone, N.L., Bellman, Z.D., Yates, A.S., England, T.J., O'Sullivan, S.E., 2019. A systematic review of cannabidiol dosing in clinical populations. Br. J. Clin. Pharmacol.

584 85, 1888–1900. https://doi.org/10.1111/bcp.14038

585 Millar, S.A., Stone, N.L., Yates, A.S., O'Sullivan, S.E., 2018. A systematic review on the
586 pharmacokinetics of cannabidiol in humans. Front. Pharmacol. 9.
587 https://doi.org/10.3389/fphar.2018.01365

588 Noblet, J., Gilbert, H., Jaguelin-Peyraud, Y., Lebrun, T., 2013. Evidence of genetic variability
589 for digestive efficiency in the growing pig fed a fibrous diet. Animal 7, 1259–1264.
590 https://doi.org/10.1017/S1751731113000463

591 O'Shea, J.P., Faisal, W., Ruane-O'Hora, T., Devine, K.J., Kostewicz, E.S., O'Driscoll, C.M.,
592 Griffin, B.T., 2015. Lipidic dispersion to reduce food dependent oral bioavailability of
593 fenofibrate: In vitro, in vivo and in silico assessments. Eur. J. Pharm. Biopharm. 96,
594 207–216. https://doi.org/10.1016/j.ejpb.2015.07.014

595 O'Shea, J.P., Nagarsekar, K., Wieber, A., Witt, V., Herbert, E., O'Driscoll, C.M., Saal, C.,
596 Lubda, D., Griffin, B.T., Dressman, J.B., 2017. Mesoporous silica-based dosage forms
597 improve bioavailability of poorly soluble drugs in pigs: case example fenofibrate. J.
598 Pharm. Pharmacol. 69, 1284–1292. https://doi.org/10.1111/jphp.12767

599 Papadopoulou, V., Kosmidis, K., Vlachou, M., Macheras, P., 2006. On the use of the Weibull
600 function for the discernment of drug release mechanisms. Int. J. Pharm. 309, 44–50.
601 https://doi.org/10.1016/j.ijpharm.2005.10.044

602 Perlstein, H., Bavli, Y., Turovsky, T., Rubinstein, A., Danino, D., Stepensky, D., Barenholz,
603 Y., 2014. Beta-casein nanocarriers of celecoxib for improved oral bioavailability. Eur. J.
604 Nanomedicine 6, 217–226. https://doi.org/10.1515/ejnm-2014-0025

605 Perucca, E., Bialer, M., 2020. Critical Aspects Affecting Cannabidiol Oral Bioavailability and
606 Metabolic Elimination, and Related Clinical Implications. CNS Drugs 34, 795–800.
607 https://doi.org/10.1007/s40263-020-00741-5

608 Rapin, L., Gamaoun, R., El Hage, C., Arboleda, M.F., Prosk, E., 2021. Cannabidiol use and
609 effectiveness: real-world evidence from a Canadian medical cannabis clinic. J.
610 Cannabis Res. 3. https://doi.org/10.1186/s42238-021-00078-w

611 Riikonen, J., Correia, A., Kovalainen, M., Näkki, S., Lehtonen, M., Leppänen, J., Rantanen,
612 J., Xu, W., Araújo, F., Hirvonen, J., Järvinen, K., Santos, H.A., Lehto, V.P., 2015.
613 Systematic in vitro and in vivo study on porous silicon to improve the oral bioavailability
614 of celecoxib. Biomaterials 52, 44–55. https://doi.org/10.1016/j.biomaterials.2015.02.014

615 Tomic, I., Mueller-Zsigmondy, M., Vidis-Millward, A., Cardot, J.M., 2018. In vivo release of
616 peptide-loaded PLGA microspheres assessed through deconvolution coupled with
617 mechanistic approach. Eur. J. Pharm. Biopharm. 125, 21–27.
618 https://doi.org/10.1016/j.ejpb.2017.12.007

619 Trevaskis, N.L., Charman, W.N., Porter, C.J.H., 2008. Lipid-based delivery systems and
620 intestinal lymphatic drug transport: A mechanistic update. Adv. Drug Deliv. Rev. 60,
621 702–716. https://doi.org/10.1016/j.addr.2007.09.007

622 Yáñez, J.A., Wang, S.W.J., Knemeyer, I.W., Wirth, M.A., Alton, K.B., 2011. Intestinal
623 lymphatic transport for drug delivery. Adv. Drug Deliv. Rev. 63, 923–942.
624 https://doi.org/10.1016/j.addr.2011.05.019

625 Zhang, Z., Quan, G., Wu, Q., Zhou, C., Li, F., Bai, X., Li, G., Pan, X., Wu, C., 2015. Loading
626 amorphous Asarone in mesoporous silica SBA-15 through supercritical carbon dioxide
627 technology to enhance dissolution and bioavailability. Eur. J. Pharm. Biopharm. 92, 28–
628 31. https://doi.org/10.1016/j.ejpb.2015.02.018

629