Data-driven development of an oral lipid-based nanoparticle formulation of a hydrophobic drug

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Methods

High-throughput SLN/NLC screening

High-throughput screening of drug-loaded SLNs/NLCs was performed on a miniaturized scale using a liquid handling robot (OT-2, Opentrons, NY, US) and 96-well plates. Each well in the plate functioned as a miniaturized reaction vessel, thereby allowing 96 experiments to be conducted per plate (i.e., 32 different formulations screened per plate, each with three experimental replicates). In order to achieve this, customized experiments were designed and validated using the OT-2 system for SLN/NLC synthesis and characterization. Each 96-well plate (Sarstedt, Germany) allows for the screening of SLNs/NLCs formulated using four solid lipid-to-liquid lipid ratios (i.e., 100:0, 90:10, 80:20, and 60:40; w/w), four initial drug-to-total lipid ratios (i.e., 5, 10, 20, and 40%, wt%), and two surfactant concentrations (i.e., 0 and 0.25% or 0.5% and 1.0%, w/v%). The total mass of lipid (solid lipid and liquid lipid) was fixed at 120 µg/well. The effect of type of solid lipid (i.e., SA, C888, or GM) on formulation performance was analyzed by screening different batches.

Experimental setup: The layout of the OT-2 liquid handling robot is shown in **Figure S1**. Each pipette tip rack (A and B) held 96 pipette tips (300 µL, Opentrons, NY, US) for SLN/NLC preparation. The deep 96-well plate (2.2 mL well volume; BRAND®, Sigma, CA) labelled A in **Figure S1** was used to prepare the various organic phases for nanoprecipitation experiments. The last two columns of the stock plate were prefilled with deionized water (with/without surfactant) as the stock of aqueous phase used for SLN/NLC formulation. An empty 96-well plate, labelled B in **Figure S2** (300 µL, Fisher Scientific, Pennsylvania, US) was used to perform the nanoprecipitation experiments, and was termed the "formulation plate". Stock solutions of drug (6 mg/mL in THF) and of the various excipients (3 mg/mL solid lipid and 10 mg/mL liquid lipid) were prepared in 20 mL scintillation vials. The solid lipid stock solutions were prepared by heating to around 50 to 70 ℃ and cooling to room temperature prior to use. These scintillation vials were then placed into a scintillation vial rack which was designed and built using a 3D printer (Creator Pro 3D printer, Flashforge, CN).

SLN/NLC preparation: Customized experimental protocols were then deployed to conduct the nanoprecipitation experiments on the OT-2. Specifically, the 8-channel pipette (300 µL) was programmed to disperse 250 µL of the aqueous phase from the stock plate to each well on the

formulation plate. Then, using the drug and excipient stock solutions in the scintillation vials, organic phases corresponding to formulations F1 to F32 (**Table S1**) were prepared in the stock plate using a single-channel pipette (300 µL). The prepared organic phases were then mixed by ten cycles of pipetting using an 8-channel pipette (300 µL). Aliquots (50 µL) of the prepared organic phases were injected into the aqueous phases (250 µL), followed by 30 cycles of pipette mixing to facilitate nanoprecipitation. The formulation plate was then placed in the fumehood overnight to allow for particle hardening. The plate was then centrifuged for 60 min at 3000 rpm to spin down drug or lipid precipitate prior to further analysis. The supernatant was then used for analysis of particle size and drug loading.

Size measurements: For size measurements, 25 µL aliquots of particles were transferred from the formulation plate to a 384-well plate (Corning® 384-well plate, Sigma, CA) using the OT-2 and an 8-channel pipette (300 µL). The 384-well plate was then centrifuged at 3000 rpm for 3 min to remove air bubbles prior to size measurements. High-throughput size measurements were performed using a dynamic light scattering (DLS) plate reader (DynaPro plate reader III, Wyatt Technology). The DLS plate reader was operated at 25 °C, 10% laser power, and a 95% attenuation level.

Drug loading analysis: Using the OT-2 and an 8-channel pipette (300 µL), 100 µL aliquots of the particles were transferred to a 96-well filter plate (100 kDa, modified polyethersulfone membrane, Pall AcroPrep Advance Filter Plates, CA) with a deep 96-well plate (0.5 mL well volume, BRAND®, Sigma, CA) attached at the bottom as a receiver. The filter plate (with the attached receiver) was then centrifuged at 3000 rpm for 60 min to separate the loaded-SLNs/NLCs from free drug (i.e., supernatant in the receiver). Following the removal of the free drug, the drug loaded particles were then reconstituted for drug analysis. For this step, the filter plate containing the SLNs/NLCs was placed on a new receiver. 200 µL of methanol was added by OT-2 using the 8-channel pipette (300 µL) to the filter plate (containing the particles), followed by 40 cycles of mixing to extract the drug. The filter plate was then centrifuged at 3000 rpm for 10 min to collect the extracted drug in the receiver. The resulting solutions containing extracted drug were then manually transferred to high performance liquid chromatography (HPLC) vials for quantification of drug loading (as described in the manuscript).

***In vitro* SLN/NLC characterization**

Fourier transform infrared (FTIR) Spectrometry: FTIR analysis was conducted to characterize and compare the functionality of the bench-scale SLNs/NLCs (freeze-dried) with the drug/excipients (starting materials). This was done using a PerkinElmer Spectrum Two FTIR Spectrometer at a spectral range of 500 to 4000 cm^{-1}. For freeze-drying, samples were frozen at -80 °C for 30 min and then dried under vacuum (FreeZone 4.5 Plus, Labconco, US) overnight prior to analysis. For FTIR analysis, samples (approximately 5 to 10 mg) were analyzed by five repeated scans using an attenuated total reflectance (ATR) accessory. ATR correction, baseline correction, and smoothing were performed on the spectra using a PerkinElmer Spectrum IR (version 10.6.2).

Differential Scanning Calorimetry (DSC): The thermal properties of bench-scale SLNs/NLCs and the drug/excipients used in formulation preparation (as received starting materials compared to freeze-dried particles) were assessed using DSC (TA Instruments Q100). Approximately 5 to 10 mg of each sample was analyzed over a temperature range of 35 to 80 °C (1 °C/min) using an empty pan as reference under sample purge flow (nitrogen) at 50 mL/min. The melting peak maximum of each sample was determined using the TA Instruments Universal Analysis 2000.

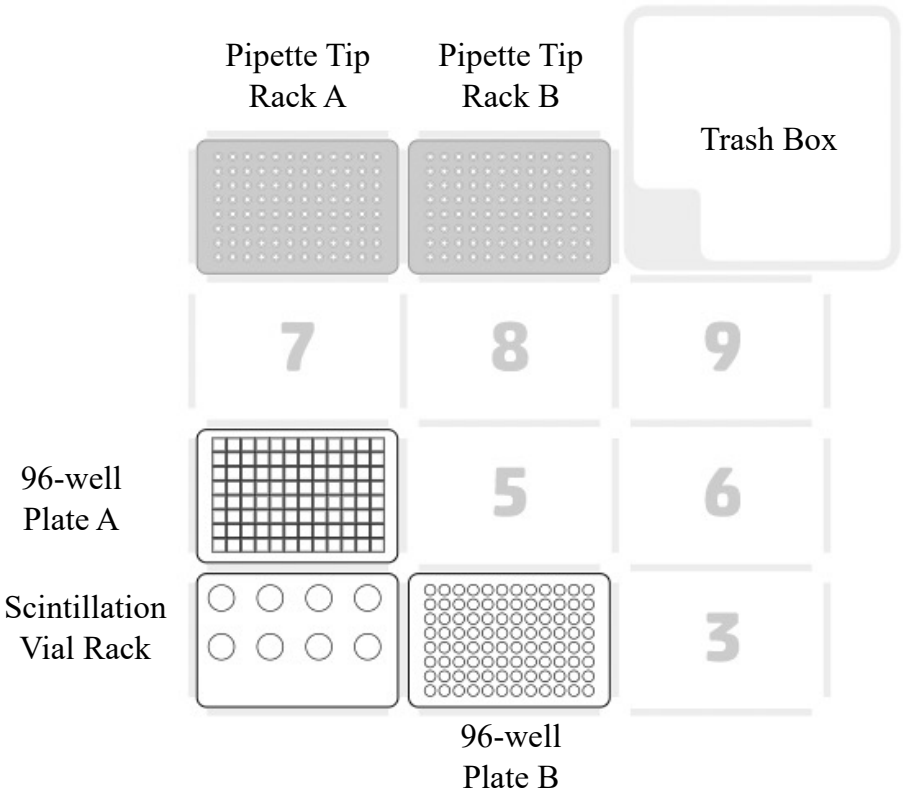


Figure S1. Overview of the labware layout in the OT-2 liquid handling robot. Each pipette tip rack (A and B) held 96 pipette tips for solid lipid nanoparticle and nanostructured lipid carrier preparation. 96-well plates A (last two columns of the stock plate were prefilled with aqueous phase) and B were used as the stock plate and formulation plate, respectively. Lab customized scintillation vial rack was used to hold drug/excipient stocks prepared in scintillation vials.

Table S1. Summary of the initial levels of drug and materials employed in the formulation of each batch of the solid lipid nanoparticles and nanostructured lipid carriers.

Formulation Codes	Solid lipid (w/w, %)	Liquid lipid (w/w, %)	Drug* (w/w, %)	Surfactant** (w/v, %)	Location in Plate (i.e., n = 3)
F1	100	0	5	0 or 0.5	A1 - A3
F2	100	0	10	0 or 0.5	A4 - A6
F3	100	0	20	0 or 0.5	A7 - A9
F4	100	0	40	0 or 0.5	A9 - A12
F5	90	10	5	0 or 0.5	B1 - B3
F6	90	10	10	0 or 0.5	B4 - B6
F7	90	10	20	0 or 0.5	B7 - B9
F8	90	10	40	0 or 0.5	B9 - B12
F9	80	20	5	0 or 0.5	C1 - C3
F10	80	20	10	0 or 0.5	C4 - C6
F11	80	20	20	0 or 0.5	C7 - C9
F12	80	20	40	0 or 0.5	C9 - C12
F13	60	40	5	0 or 0.5	D1 - D3
F14	60	40	10	0 or 0.5	D4 - D6
F15	60	40	20	0 or 0.5	D7 - D9
F16	60	40	40	0 or 0.5	D9 - D12
F17	100	0	5	0.25 or 1.0	E1 - E3
F18	100	0	10	0.25 or 1.0	E4 - E6
F19	100	0	20	0.25 or 1.0	E7 - E9
F20	100	0	40	0.25 or 1.0	E9 - E12
F21	90	10	5	0.25 or 1.0	F1 - F3
F22	90	10	10	0.25 or 1.0	F4 - F6
F23	90	10	20	0.25 or 1.0	F7 - F9
F24	90	10	40	0.25 or 1.0	F9 - F12
F25	80	20	5	0.25 or 1.0	G1 - G3
F26	80	20	10	0.25 or 1.0	G4 - G6
F27	80	20	20	0.25 or 1.0	G7 - G9
F28	80	20	40	0.25 or 1.0	G9 - G12
F29	60	40	5	0.25 or 1.0	H1 - H3
F30	60	40	10	0.25 or 1.0	H4 - H6
F31	60	40	20	0.25 or 1.0	H7 - H9
F32	60	40	40	0.25 or 1.0	H9 - H12

** Drug content was determined as the ratio of the mass of drug input to the mass of total lipid*

*** Surfactant concentration was determined as the ratio of the mass of surfactant to the volume of the aqueous phase. 0.5% and 1.0% surfactant concentrations were investigated only for Compritol particles.*

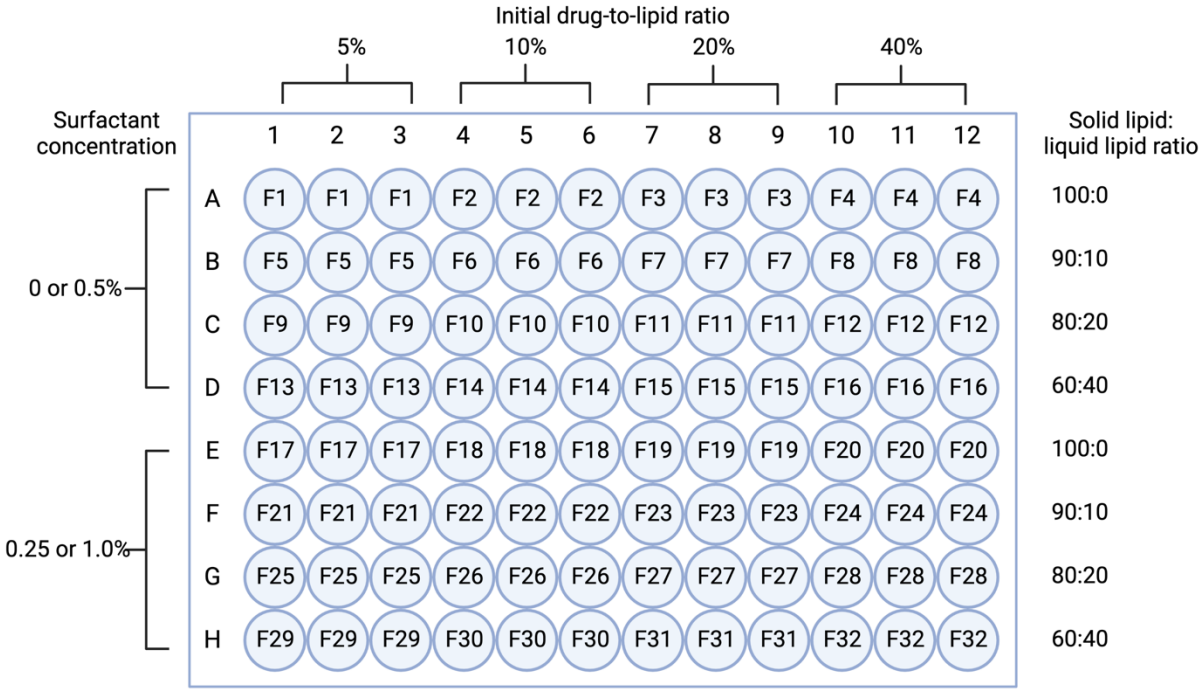


Figure S2. Description of the experimental layout for each 96-well plate with the location and formulation codes for each formulation screened. Specifically highlighted are the initial drug-to-lipid ratios (wt%), solid lipid:liquid lipid ratios (w:w), and concentration of surfactant in aqueous phase (w/v, %).

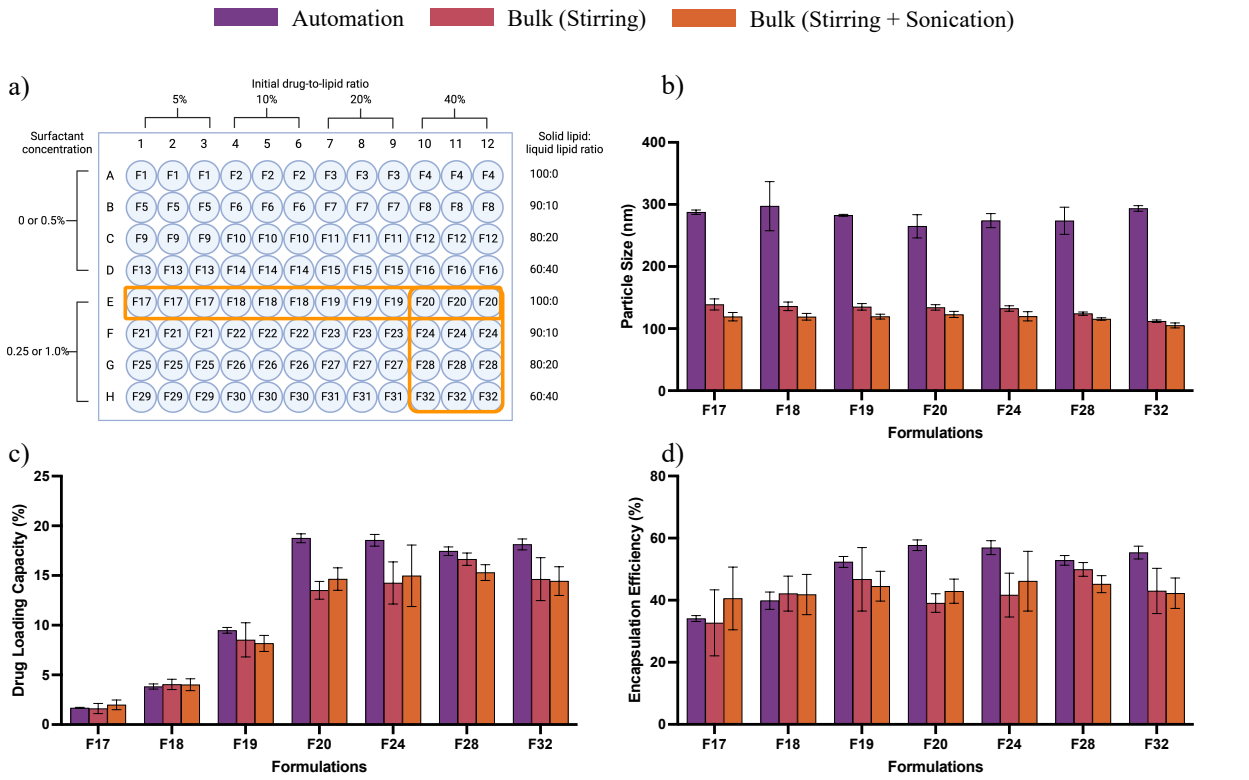


Figure S3. Comparison of the solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) prepared using the automated workflow within a 96 well plate (as highlighted in Figure a) and the bulk preparation methods (stirring +/- sonication) in terms of SLN/NLC size (b), drug loading capacity (DLC, c), and drug encapsulation efficiency (EE, d). The SLNs/NLCs (made using Compritol as the solid lipid and 0.25% surfactant) were prepared as outlined in the manuscript. To investigate the effects of sonication on formulation properties, half of the particles (i.e., 9 mL) were transferred to another 20 mL scintillation vial and sonicated (sonication probe; Fisher Scientific model 100) for 10 min while stirring (400 rpm).

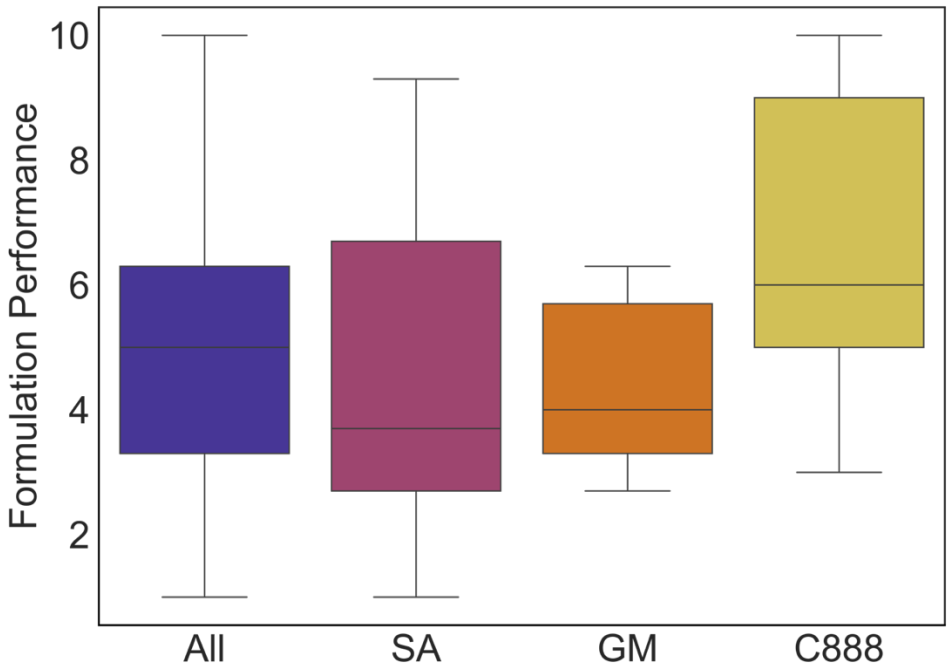


Figure S4. The overall formulation performance of the particles in the design space (~1200 formulations) compared with a breakdown of formulation performance for nanoparticles formulated using distinct types of solid lipids.

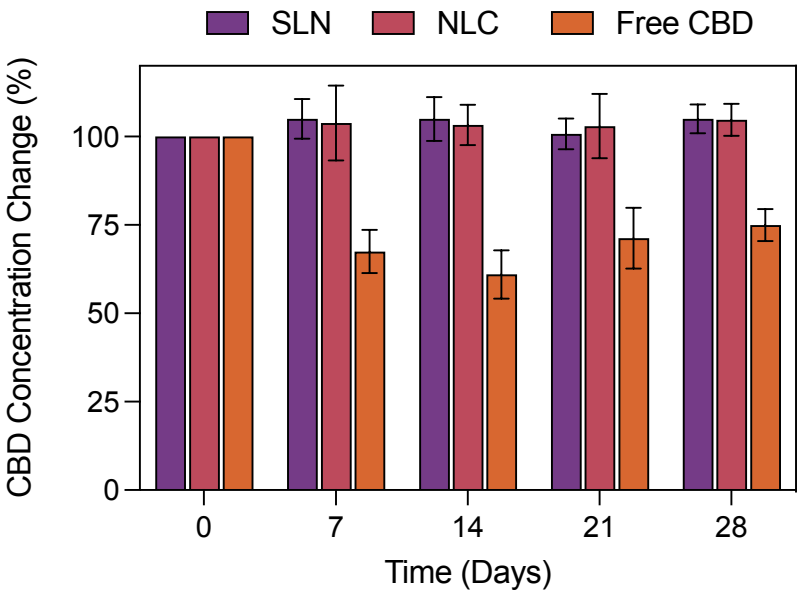


Figure S5. Stability of CBD in the solid lipid nanoparticle and nanostructured lipid carrier formulations. Drug concentrations were measured at the start of the study (D0) and samples were stored at room temperature in darkness. Samples were taken every seven days until 28 days (D28) for evaluation of CBD stability. The drug concentrations on D0 were set as control (i.e., 100%), concentration changes were calculated as the ratio of drug concentration compared to the control (D0 concentrations). Free drug (dissolved in 0.5% Tween 80, v/v) was examined in the same way for comparison.

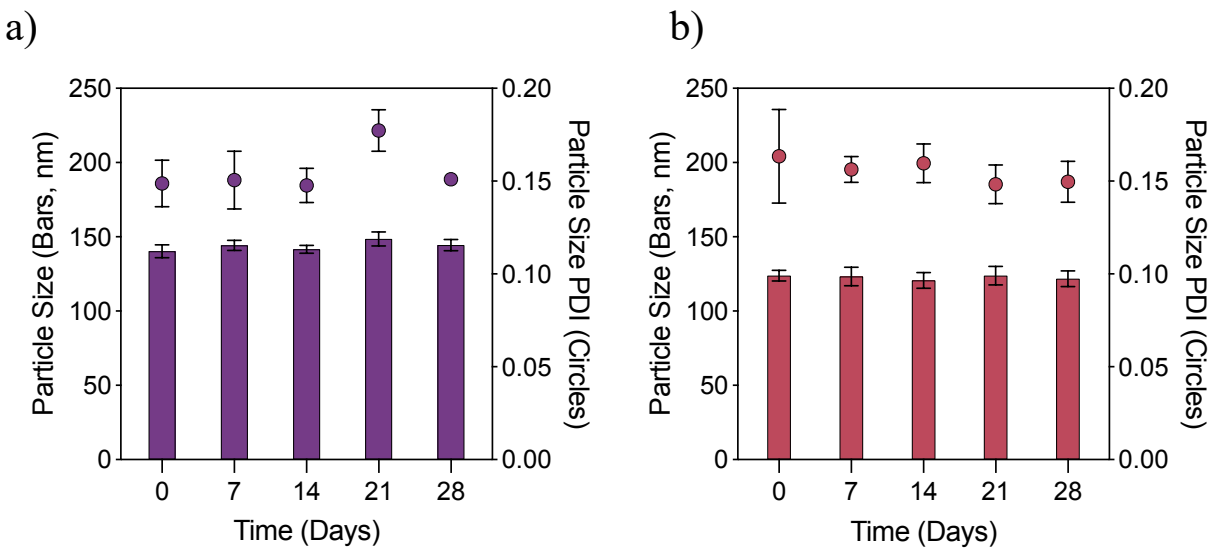


Figure S6. a) and b) show the particle size and PDI of the identified solid lipid nanoparticle and nanostructured lipid carrier, respectively, and their change over one month during storage at room temperature in darkness.

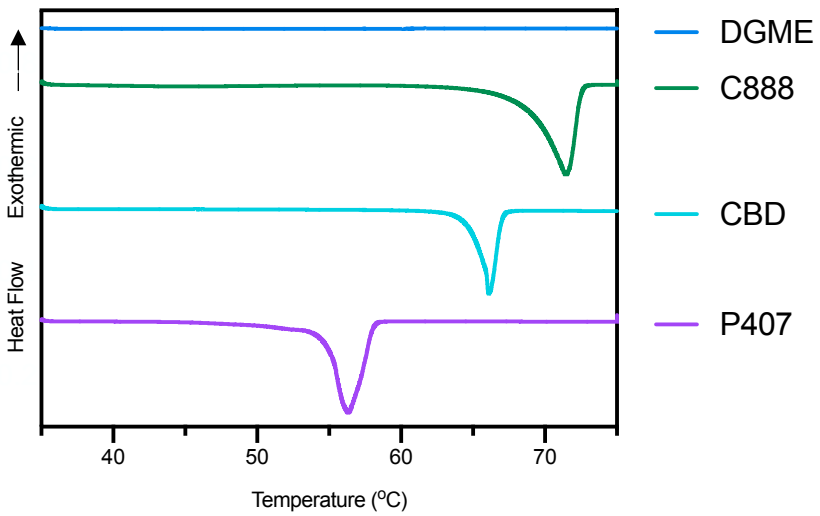
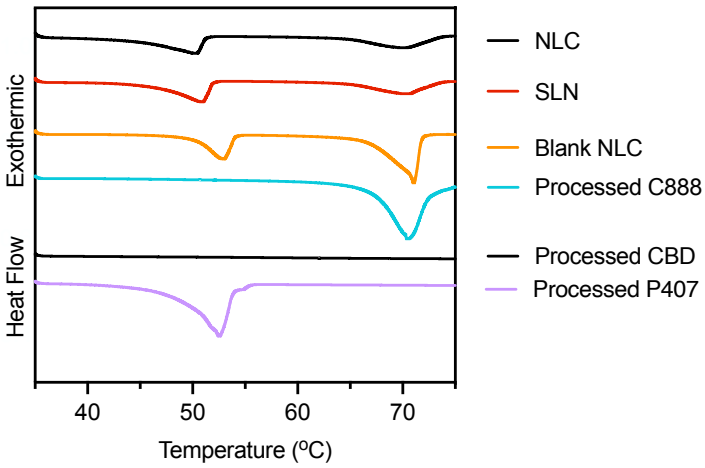


Figure S7. Differential scanning calorimetry (DSC) diagrams of the starting materials (as received) used for preparation of the identified formulations.

a)

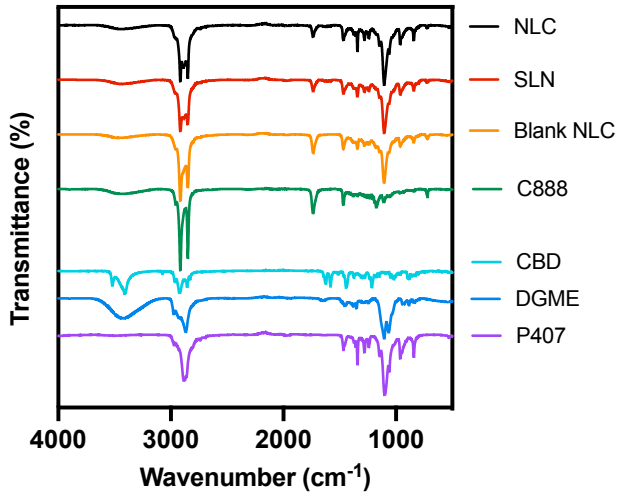


b)

Composition	C888	CBD	DGME	P407
NLC	+	+	+	+
SLN	+	+	-	+
Blank NLC	+	-	+	+

Figure S8. a) Differential scanning calorimetry (DSC) thermograms of the identified solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC) formulations. DSC thermograms of blank NLC, unformulated drug, and excipients (processed in the way particles were formulated) are included for comparison. b) A summary of the composition of SLN, NLC, and blank NLC. By comparing SLN and NLC, the effects of liquid lipid DGME can be illustrated. By comparing NLC and blank NLC, the effects of drug can be illustrated.

a)



b)

Composition	C888	CBD	DGME	P407
NLC	+	+	+	+
SLN	+	+	-	+
Blank NLC	+	-	+	+

Figure S9. a) Fourier transform infrared (FTIR) spectra of the identified solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC) formulations. FTIR spectra of blank NLC, unformulated drug, and excipients are included for comparison. By comparing SLN and NLC, the effects of liquid lipid DGME can be illustrated. By comparing NLC and blank NLC, the effects of drug CBD can be illustrated.

Table S2. Gradient elution details for the detection of CBD and THC (internal standard) by HPLC-MS in rat serum samples. The mobile phase consisted of water (A) and ACN (B), with both A and B phases containing 0.1% (v/v) formic acid.

Time (min)	Flow rate (mL/min)	Mobile Phase A (%)	Mobile Phase B (%)
0	0.25	35	65
1	0.25	5	95
4	0.25	5	95
4.1	0.25	35	65
10	0.25	35	65

Table S3. Parameters used for HPLC-MS analysis of CBD in rat serum samples.

Parameter	Value
Sheath gas (arb)	25
Aux gas (arb)	10
Sweep gas (arb)	2.8
Ion transfer tube temp (°C)	275
Vaporizer temp (°C)	150
Positive ion voltage (V)	4000
Negative ion voltage (V)	3500

Table S4. Retention time and other relevant parameters for the detection of CBD and THC (internal standard) by HPLC-MS in rat serum samples.

Drug	Retention Time (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)
CBD	3.25	Positive	315.0	245.0	20
CBD	3.25	Positive	315.0	193.0	20
THC	4.10	Positive	315.0	259.0	24
THC	4.10	Positive	315.0	193.0	24

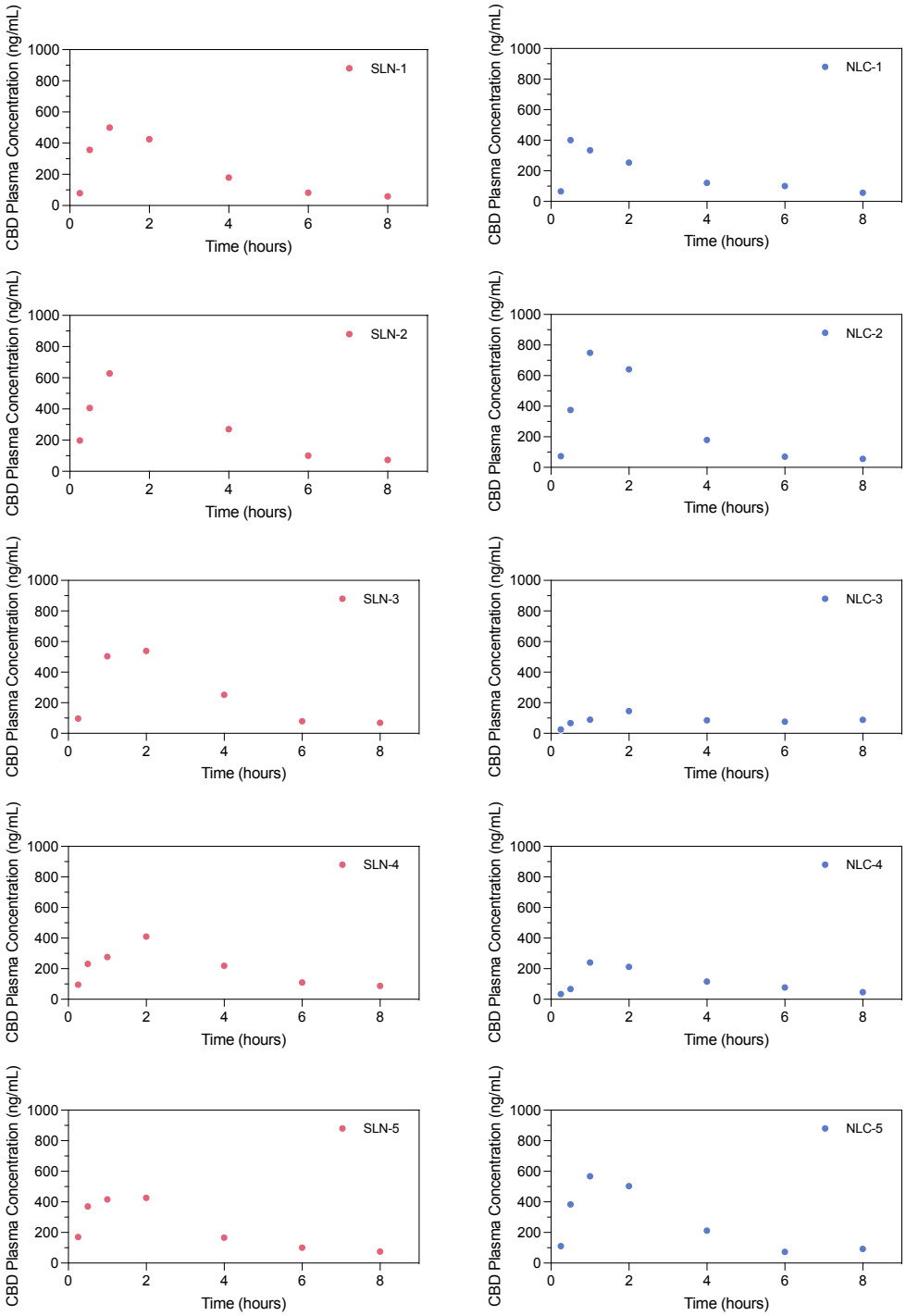


Figure S10. Individual plots of the drug plasma concentrations (five rats for each formulation) post solid lipid nanoparticle and nanostructured lipid carrier administration in Sprague Dawley rats at 20 mg/kg CBD. Blood samples were taken at predetermined timepoints, followed by centrifuge for plasma separation from the whole blood. Drug concentrations in plasma were analyzed using liquid chromatography-mass spectrometry (LC-MS).