Insect larvae oil: a high value excipient for lipid-based nanocarriers to tackle atopic dermatitis

Cíntia Almeida[1,2], Rossana Roque[1], João Vieira[1,2], Ana Júlio[1], Nuno Saraiva[1], Catarina Pereira-Leite[1,3*], Catarina Rosado[1*]

[1] CBIOS - Universidade Lusófona's Research Center for Biosciences & Health Technologies, Lisboa, Portugal
[2] Universidad de Alcalá, Departamento de Ciências Biomédicas, Madrid, Spain
[3] LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

**catarina.rosado@ulusofona.pt; catarina.leite@ulusofona.pt – shared senior authorship*

Abstract:

Atopic dermatitis (AD) is a chronic inflammatory skin disorder with a complex pathogenesis involving epidermal barrier dysfunction and aberrant lipid composition, particularly ceramides and fatty acids (FA). Conventional management options, such as topical glucocorticoids (GC), often lead to adverse effects upon prolonged usage, necessitating the exploration of alternative therapeutic strategies. This study investigated the potential of utilizing novel nanotechnology-based formulations to enhance the topical management of AD. Specifically, we explored the use of solid lipid nanoparticles (SLN) formulated with insect larvae oil as a carrier for dexamethasone (DEX), a representative GC, and as an adjunctive emollient to support skin barrier repair. The lipidic fraction of Black Soldier Fly larvae biomass, holding a rich blend of FA, holds substantial potential as a novel ingredient to tackle skin barrier impairment. Through systematic optimization using Box-Behnken Design, insect larvae oil-based SLN demonstrated favorable physicochemical properties for topical application and satisfactory stability over 2 months. Notably, these SLN exhibited a favorable drug release kinetics, delivering the total DEX payload within a therapeutically relevant timeframe. Furthermore, these SLN showed ability to permeate human keratinocytes without pronounced toxicity, suggesting their potential utility in enhancing drug delivery and cellular uptake. Overall, our findings suggest that insect larvae oil is a promising natural and sustainable ingredient for the development of nanotechnology-driven approaches to AD management, offering a potential avenue for addressing the unmet needs in this challenging dermatologic condition.

Keywords: Solid lipid nanoparticles; Box-Behnken design; Skin barrier; Topical delivery systems, Glucocorticoids.

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

1. Introduction

Atopic dermatitis (AD) presents a significant threat among skin conditions, consistently contributing to patient disability, with its prevalence continuing to escalate (1, 2). A hallmark of AD is the compromised epidermal barrier, primarily stemming from an aberrant lipid metabolism, leading to ceramide and fatty acid (FA) profile disturbances in the *stratum corneum* (3-5). The gold standard for AD management involves daily application of topical emollients coupled with either reactive or proactive topical use of glucocorticoids (GC) or calcineurin inhibitors (6). Dexamethasone (DEX) is a widely used glucocorticoid to treat inflammatory skin dysfunctions, such as atopic dermatitis. Despite being considered a low potency glucocorticoid (7), there are some adverse effects associated with its topical application and limitations to its transcutaneous penetration.

Nanotechnology can provide different tools for developing innovative carriers, such as lipid nanoparticles for the delivery of drugs. Solid lipid nanoparticles (SLN) provide advantages like bio-vectorization and improved bioavailability, biocompatibility and biodegradability (3, 8). In this sense, these encapsulation systems can facilitate the permeation of drugs, as well as the protection of these compounds from possible physicochemical alterations or degradations (8). These characteristics can be used in topical formulations, providing an optimized release to the skin, as well as an improved delivery to the therapeutic target, thereby reducing the occurrence of adverse effects.

The *stratum corneum* (SC) is the outermost epidermal layer, providing an efficient barrier for many xenobiotics, including topical formulations. The SC is formed by corneocytes, mainly composed of keratin, immersed in an impermeable lipid matrix. Several skin diseases are related to alterations in this lipidic medium, causing dysfunctions in the cutaneous barrier and resulting in clinical manifestations as inflammation processes, including atopic dermatitis and psoriasis (3, 4). In addition to SC ceramides and cholesterol, free fatty acids (FA) perform a critical role in skin barrier function and in the regulation of water loss. The development of lipid nanoparticles made up of FA that are similar to those present in the epidermis may, thus, be a good strategy for topical delivery, since they can combine optimized delivery with an emollient protective effect.

Sustainable technological approaches are hot topics in R&D&I and in current European Union and United Nations development policies. Moreover, the need has arisen in Europe to increase its resilience and explore locally sourced biomaterials as the foundation for innovative formulations. *Hermetia illucens* larvae biomass can be viewed as a source of compounds with high aggregated value and marketing potential due to the sustainable organic matter bioconversion process used as substrate for its development. The larvae biomass of the Black Soldier Fly (BSF) is known to be rich in proteins, vitamins, minerals and, most importantly, lipids (9). BSF larvae lipid content is mainly composed of saturated FA, namely lauric acid (C12:0), followed by palmitic (C16:0), linoleic (C18:2), and oleic (C18:1n-9) acids (10). This blend of FA of BSF larvae biomass has a great potential as a new and sustainable ingredient and may be applied in cutaneous nanotechnology-based formulations as an emollient raw material (9).

In this study, an innovative strategy for DEX skin nanodelivery was attempted using a sustainable, natural and versatile raw material. SLN based on the BSF lipid extract were designed for the treatment of atopic dermatitis, to ultimately provide both controlled release and emollient properties. The optimized DEX-loaded SLN was developed via a Box-Behnken Design (BBD) approach and further physicochemically characterized in terms of storage stability and *in vitro* drug release. The SLN impact on HaCaT cell viability was assessed by an MTT test and an internalization assay was performed to

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

evaluate the nanoparticles' capacity to permeate these cells. Overall, this proof-of-concept study aims to establish insect larvae oil as a sustainable and versatile ingredient to produce glucocorticoid-loaded nanoformulations to tackle AD.

2. Materials and Methods

2.1 Materials

The BSF larvae were kindly supplied by Entogreen® company. The lipid extract was obtained from their biomass with a maceration technique using an organic solvent, as described by Almeida et al. (10). DEX was obtained from AppliChem GmbH (Darmstadt, Germany) and Tween® 80 from Sigma-Aldrich (Saint Louis, MO, USA).

2.2 SLN preparation

SLN were prepared by hot homogenization followed ultrasonication using a Q125 Sonicator (QSonica Sonicators, Newtown, CT, USA). Briefly, the lipid extract was mixed with Tween® 80 (lipid phase) and melted in a water bath at 50 °C. Distilled water was heated to the same temperature of the lipid phase. After melting the lipid extract, the heated distilled water was added to the lipid phase, followed by ultrasonication (70% amplitude for 5 minutes). The resulting formulation was then cooled at room temperature.

A preliminary screening for the preparation of SLN made up of larvae lipid extract (5% w/v) was performed using different amounts of surfactant (60 mg or 80 mg).

For the preparation of DEX-loaded SLN, the maximum solubility of the drug in the BSF lipid extract was determined. During the SLN preparation, the drug was dissolved in the lipid phase and all other steps of the procedure were maintained.

2.3 SLN physicochemical characterization

The particle size (PS), polydispersity index (PDI), and zeta potential (ZP) of the SLN formulations was determined using the NanoBrook Omni equipment (Brookhaven Instruments, Holtsville, NY, USA). The PS and PDI measurements were run by dynamic light scattering (DLS), consisting of three reading cycles of 60 s. The ZP analyses were performed considering one run of 30 reading cycles by phase-analysis light scattering (PALS). For the analysis, an aliquot of the formulations was diluted in distilled water (50x). The measurements were conducted at 25 °C and in triplicate.

The encapsulation efficiency (EE) and loading capacity (LC) of the SLN were estimated by measuring the non-loaded fraction of DEX after employing an ultrafiltration-centrifugation technique. First, DEX-loaded SLN were diluted 1:1 (v/v) with distilled water and transferred to VIVASPIN® 500 centrifugation devices (50 KDa, Sartorius, Goettingen, Germany). The samples were then centrifuged (Hermle Z323K centrifuge, Hermle LaborTechnik, Wehingen, Germany) at 12,000 ×g for 60 min. After that, the supernatant that moved through the filter membrane was diluted in a water:ethanol solution (75:25). The non-loaded fraction of DEX was determined by recording the DEX absorbance at 241 nm in a UV/Visible spectrophotometer (Evolution® 300, Thermo Scientific, Hertfordshire, UK), considering the DEX calibration curve obtained in the same

https://doi.org/10.26434/chemrxiv-2024-3fw8h ORCID: https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. License: CC BY-NC-ND 4.0

solvent mixture. The EE and LC were finally calculated according to the equations 1 and 2.

$$EE(\%) = \frac{([\mathrm{DEX}_T] - ([DEX_{UL}])}{[DEX_T]}, \quad (1)$$

$$LC(\%) = \frac{([\mathrm{DEX}_T] - ([DEX_{UL}])}{[EXL_T]}, \quad (2)$$

where $[DEX_T]$ is the total concentration of DEX in the formulation, $[DEX_{UL}]$ is the concentration of unloaded DEX quantified in the filtrate, and $[EXL_T]$ is the total concentration of lipid extract used to formulate the nanoparticles.

2.4 SLN optimization by Box-Behnken design

The optimization of SLN formulations was based on a Design-of-Experiments (DOE) strategy by applying a 15-runs, 3-factors, 3-levels Box-Behnken design with the aim to achieve the best combination of independent variables to produce an optimized formulation for topical delivery.

The amount of surfactant, lipid extract and DEX were selected as factors (independent variables) and PS, PDI, ZP, EE and LC as responses (dependent variables). From the results of preliminary studies, three levels per factor were established to be tested (Table 1). The desirable values to be obtained for each response are also presented in Table 1. After mathematically analyzing the data obtained with 15 SLN formulations using the Statistica® software (Statsoft, Tulsa, OK, USA), the optimum level for each factor to achieve the higher desirability level was predicted. The composition of the optimized SLN formulation (OF) was validated by preparing the OF in triplicate and by comparing the experimentally obtained physicochemical properties (PS, PDI, ZP, EE and LC) with the theoretical ones predicted by the statistical analysis.

Table 1. Independent variables and corresponding levels chosen for the Box-Behnken Design (BBD). Dependent variables and the selected desirability criteria are also presented.

Variables	**Levels**		
	-1	**0**	**1**
X1: Surfactant (mg)	60	70	80
X2: Extract (mg)	100	125	150
X3: DEX (mg)	1.0	1.30	1.50
	Desirability		
	Low	**Medium**	**High**
Y1: PS (nm)	250	175	100
Y2: PDI	0.30	0.25	0.20
Y3: ZP (mV)	-30	-38	-45
Y4: EE (%)	60	75	90
Y5: LC (%)	0.50	0.85	1.20

PS: particle size; PDI: polydispersity index; ZP: zeta potential; EE: encapsulation efficiency; LC: loading capacity.

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

2.5 SLN storage stability

The storage stability of the optimized SLN was assessed by maintaining the formulation at 25 ± 3 °C for 60 days, according to ICH guidelines (11), and by characterizing it in terms of PS, PDI, ZP, EE, LC, and pH at specific time-points (7, 14, 30, 45, and 60 days) after the preparation day. All measurements were performed in triplicate and according to the specifications described in section 2.3. pH values were obtained using a 744 pH meter (Metrohm AG, Herisau, Switzerland) after calibration with standard solutions at room temperature.

2.6 In vitro drug release

In vitro release studies of the DEX from the optimized SLN were performed based on a dialysis bag diffusion method. The dialysis membrane (Spectra/Por™ 1, MWCO 6-8 KD, Spectrum™, Rancho Dominguez, CA, USA) was pre-hydrated in distilled water for about 30 min. The receptor compartment, containing 80 ml of a mixture of phosphate buffer saline (pH 7.4) and ethanol (75:25), was kept at 37 ± 2 °C and stirred with a magnetic stirring bar throughout the experiment. The optimized SLN formulation (1.75 ml) was introduced in the dialysis membrane, in triplicate, and placed in the receptor medium. At pre-determined time intervals, aliquots (2 ml) were taken out from the receptor compartment and replaced by an equal volume of fresh receptor solution to ensure the sink conditions. The withdrawn samples were suitably diluted, and the analyses of the cumulative drug release were done using the calibration curve method at 241 nm using a UV/Vis spectrophotometer (Evolution® 300, Thermo Scientific, Hertfordshire, UK).

2.7 Cell culture and in vitro cytotoxicity

The cytotoxicity of DEX, the lipid extract, unloaded SLNs, and DEX-loaded SLNs was evaluated using the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay. The human keratinocyte HaCaT cell line was used as model. The cells were maintained at 37 °C in an 5% CO_2 atmosphere and cultured in DMEM low-glucose medium (Biowest) supplemented with 10% Fetal Bovine Serum (Enzyfarma) and 2% Penicillin-streptomycin (Enzyfarma). For the viability test, cells were seeded in 96-well plates at a density of $1x10^4$ cells/well and were grown for 24 h. Each compound or formulation to be tested, at different concentrations (corresponding to 0.01-100 µM of DEX) was added and left to incubate for 24 h. A DMSO (final concentration 5%) was used as positive control for cell death and water or a water:DMSO solution were used as vehicle controls. Cells were incubated with MTT (0.5 mg/mL) for 2 h, washed with PBS and the resulting formazan was solubilized with DMSO. The absorbances were measured at 595 nm in a microplate reader (Synergy HTX, BioTek Instruments, Bad Friedrichshall, Germany) and cells incubated with vehicle controls were considered 100% viable.

2.8 Internalization assay

Fluorescein isothiocyanate (FITC)-loaded SLN were prepared by dissolving the fluorescent probe (1 mg) in the lipid phase and all other steps of the SLN production procedure remained the same. After preparation, FITC-loaded SLN were submitted to

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

dialysis (Spectra/Por™ 1, MWCO 6-8 KD, Spectrum™, Rancho Dominguez, CA, USA) in PBS for 24 h to remove the non-encapsulated fraction of FITC.

To evaluate the SLN capacity to permeate HaCaT cells, a cellular uptake assay was performed. Cells were seeded in 12-well plates at a density of $3x10^5$ cells/well. After 24 h, FITC-loaded and unloaded SLN were added to the wells and left to incubate for 4 h. PBS and the last wash from FITC-loaded SLNs dialysis were added to cells to determine the amount of cell fluorescence that might arise from soluble, rather than from encapsulated FITC. After 4 h, the cells were washed twice with PBS, detached with 0.05% trypsin-EDTA (ThermoFisher), washed again twice with PBS and analyzed by flow cytometry on the FACSCalibur™ Flow Cytometer (BD). A minimum of 10,000 events were recorded for each sample. Data acquisition and analysis were performed using the CellQuest (BD) and FlowJo software (Tree Star, San Carlos, CA, USA), respectively.

2.9 Statistical analysis

The statistical analysis of the data obtained from the Box-Behnken Design was conducted using STATISTICA® software (Statsoft, Tulsa, OK, USA). Data relating to characterization, stability, and cytotoxicity were analyzed with GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). Statistical differences were assessed through two-way ANOVA, followed by Tukey's multiple comparison tests. Results were considered statistically significant when p-values < 0.05.

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

3. Results

3.1 Optimization of insect larvae oil-based SLN

3.1.1 Preliminary screening

Aiming to evaluate the feasibility to produce larvae lipids-containing SLN and to establish the optimal concentration of surfactant to be used, SLNs were prepared with 60 mg and 80 mg of Tween® 80 in the preliminary screening.

The characterization studies showed that, in this first attempt, it was possible to produce unloaded and DEX-loaded SLN with very promising physicochemical properties (Table 2). When comparing equivalent surfactant concentrations, the two types of SLN had similar characteristics. A higher amount of surfactant resulted in lower PS and higher ZP. The EE and LC of the formulations were improved when 80 mg of surfactant was used. These results were on the origin of the selected independent variables and respective levels to test in the quality-by-design approach.

Table 2. Preliminary results of unload and DEX-loaded SLN prepared with different surfactant concentrations.

Samples	**Surfactant quantity** (mg)	**PS** (nm)	**PDI**	**ZP** (mV)	**EE** (%)	**LC** (%)
U_SLN	60	155 ± 1	0.24 ± 0.02	-47 ± 2	-	-
DEX_SLN		164 ± 5	0.23 ± 0.02	-49 ± 1	74.2	0.95
U_SLN	80	121 ± 1	0.24 ± 0.01	-42 ± 2	-	-
DEX_SLN		107 ± 2	0.24 ± 0.01	-42 ± 1	84.0	0.92

U_SLN: unloaded nanoparticles; DEX_SLN: DEX-loaded nanoparticles.

3.1.2 Box-Behnken design

Based on the preliminary results, three main independent variables (factors) - surfactant, extract, and DEX quantities - were selected for the implementation of BBD to optimize the larvae lipid extract-containing SLN. Three levels were tested for each independent variable, resulting in fifteen combinations of nanoparticles to be produced and characterized. The obtained results are shown in Table 3.

The formulation was optimized based on the desirability criteria defined – PS, PDI and ZP values should be minimized to ensure the adequate properties for skin permeation and colloidal stability, and EE and LC values should be maximized to achieve the best possible DEX encapsulation. To validate the optimization procedure, a SLN formulation was prepared using the optimal levels of factors. The composition of the optimized formulation, and the predicted and experimental responses gathered are shown in Table 4. The experimental values were similar to the theoretical values, validating the quality-by-design strategy.

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

Table 3. Composition of the fifteen BBD formulations and their physicochemical properties.

#	Factors			Responses				
	Surfactant (mg)	Extract (mg)	DEX (mg)	PS (nm)	PDI	ZP (mV)	EE (%)	LC (%)
1	60	100	1.25	174 ± 2	0.22 ± 0.01	-37.2 ± 0.9	83.9 ± 0.1	1.09 ± 0.01
2	80	100	1.25	107 ± 3	0.26 ± 0.01	-35.8 ± 0.8	71.8 ± 0.9	0.93 ± 0.01
3	60	150	1.25	248 ± 4	0.26 ± 0.00	-43 ± 5	82 ± 1	0.71 ± 0.01
4	80	150	1.25	205 ± 2	0.27 ± 0.02	-42 ± 2	82.9 ± 0.8	0.72 ± 0.01
5	60	125	1.0	215 ± 3	0.26 ± 0.01	-44 ± 2	64 ± 2	0.51 ± 0.01
6	80	125	1.0	169 ± 2	0.24 ± 0.01	-35 ± 2	78 ± 2	0.62 ± 0.02
7	60	125	1.5	222 ± 5	0.25 ± 0.01	-42 ± 1	85 ± 1	1.01 ± 0.01
8	80	125	1.5	163 ± 1	0.24 ± 0.01	-35 ± 4	83.2 ± 0.5	1.00 ± 0.01
9	70	100	1.0	126 ± 1	0.24 ± 0.00	-37 ± 1	68 ± 2	0.68 ± 0.02
10	70	150	1.0	217 ± 2	0.25 ± 0.01	-39.9 ± 0.3	76.6 ± 0.1	0.51 ± 0.01
11	70	100	1.5	150 ± 1	0.22 ± 0.01	-38 ± 3	82.3 ± 0.8	1.23 ± 0.01
12	70	150	1.5	249 ± 11	0.27 ± 0.01	-41 ± 2	89.5 ± 0.7	0.89 ± 0.01
13	70	125	1.25	184 ± 4	0.24 ± 0.01	-35 ± 2	81 ± 2	0.84 ± 0.02
14	70	125	1.25	178 ± 1	0.25 ± 0.01	-37.4 ± 0.7	78 ± 1	0.81 ± 0.01
15	70	125	1.25	181 ± 1	0.24 ± 0.01	-36.3 ± 0.2	79.3 ± 0.4	0.82 ± 0.01

Table 4. Predicted and experimental results for the optimal formulation. Data are expressed as mean ± standard deviation (n = 4).

Optimized Formulation		**PS (nm)**	**PDI**	**ZP (mV)**	**EE (%)**	**LC (%)**
Surfactant/ Extract/ DEX (mg) 65/100/1.50	Predicted values	167.3	0.21	-37.6	87.05	1.26
	Experimental responses	172 ± 2	0.25 ± 0.01	-36 ± 2	83.8 ± 0.9	1.27 ± 0.01

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

3.2 Storage stability of insect larvae oil-based SLN

To evaluate stability and confirm that all properties were maintained during storage, both unloaded and DEX-loaded optimized formulations were characterized for 60 days at room temperature (25 ± 3 °C). Overall, SLN showed good stability and kept satisfactory physicochemical characteristics for PS, PDI, ZP, pH, EE and LC (Figure 1). Only a discrete variation of the pH was observed for unloaded formulations from the day 14, but within adequate values for cutaneous application (pH 4 to 6). The values for EE were kept around 80% and for LC around 1.2% during the stability evaluation.

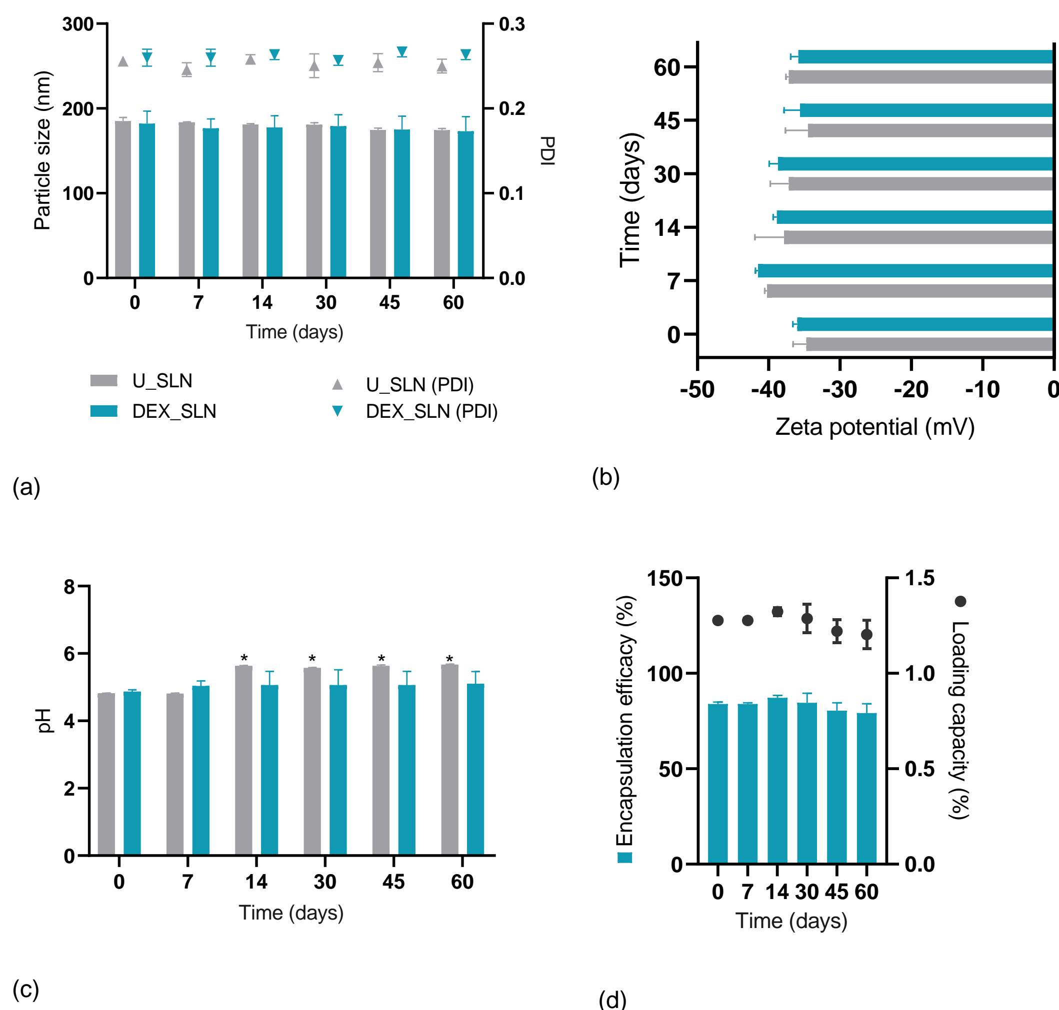

*Figure 1. Physicochemical characteristics of unloaded and DEX-loaded SLN evaluated for 60 days upon storage at room temperature. (a) Particle size and polydispersity index (PDI); (b) zeta potential (ZP); (c) pH; (d) encapsulation efficiency (EE) and loading capacity (LC). Data are showed as mean ± standard deviation (n = 3) and significant differences compared to initial data (t = 0 days) are identified with * (p-values <0.05).*

3.3 In vitro drug release of insect larvae oil-based SLN

In order to evaluate the delivery performance of the optimized formulation, an *in vitro* drug release study was performed. The cumulative percentage of DEX release into the receptor phase was investigated for a period of 26 h (Figure 2). After 15 h, the entrapped DEX in the solid lipid matrix had been fully released.

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

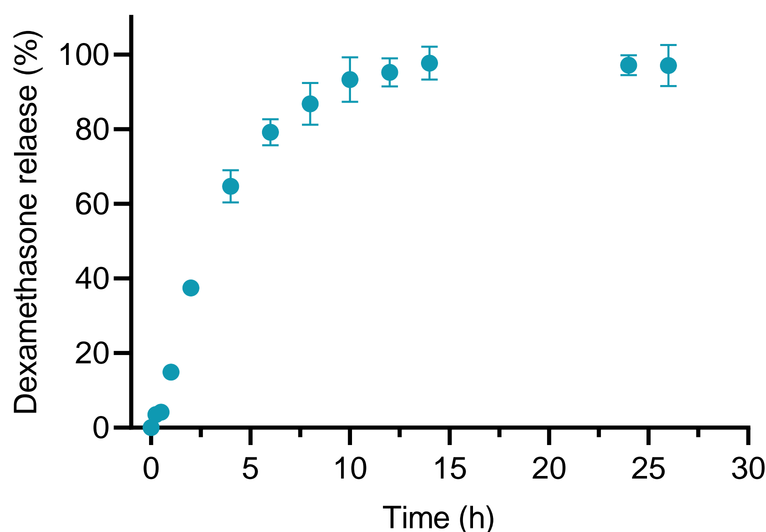

Figure 2. Dexamethasone release profile from the optimized SLN formulation at pH 7.4 (mean ± standard deviation, n = 3).

3.4 Cytotoxicity and cellular uptake of insect larvae oil-based SLN

The impact of DEX, lipid extract, unloaded SLN, and DEX-loaded SLN on the viability of HaCaT cells was assessed after a 24 h incubation using the MTT assay. The results obtained for the tested compounds or formulations are presented in Figure 3. The cells treated with a toxic concentration of DMSO (5%) had a significant reduction in viability compared to the vehicle control cells, as expected. Exposure to DEX or to the lipid extract did not cause significant cytotoxicity at the conditions tested. The viability of human keratinocytes was maintained for concentrations corresponding to 50 µM of DEX for unloaded SLN and to 10 µM of DEX for loaded SLN. For higher concentrations of insect larvae oil-based SLN a reduction of viability was observed in these conditions.

The ability of the nanoparticles to deliver their content into cells was assessed by measuring the fluorescence of individual cells after incubation with FITC-loaded SLN. The average fluorescence of cells incubated with a number of nanoparticles corresponding to 1250 and 2500 mg/L of lipid extract was 13 and 30 times higher, respectively, when compared with the fluorescence of cells incubated with unloaded nanoparticles (Figure 3 (d)). Cells incubated with PBS or the soluble FITC remaining after nanoparticle dialysis presented the lower level of fluorescence, thus attesting that possible traces of soluble FITC do result in cell fluorescence.

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

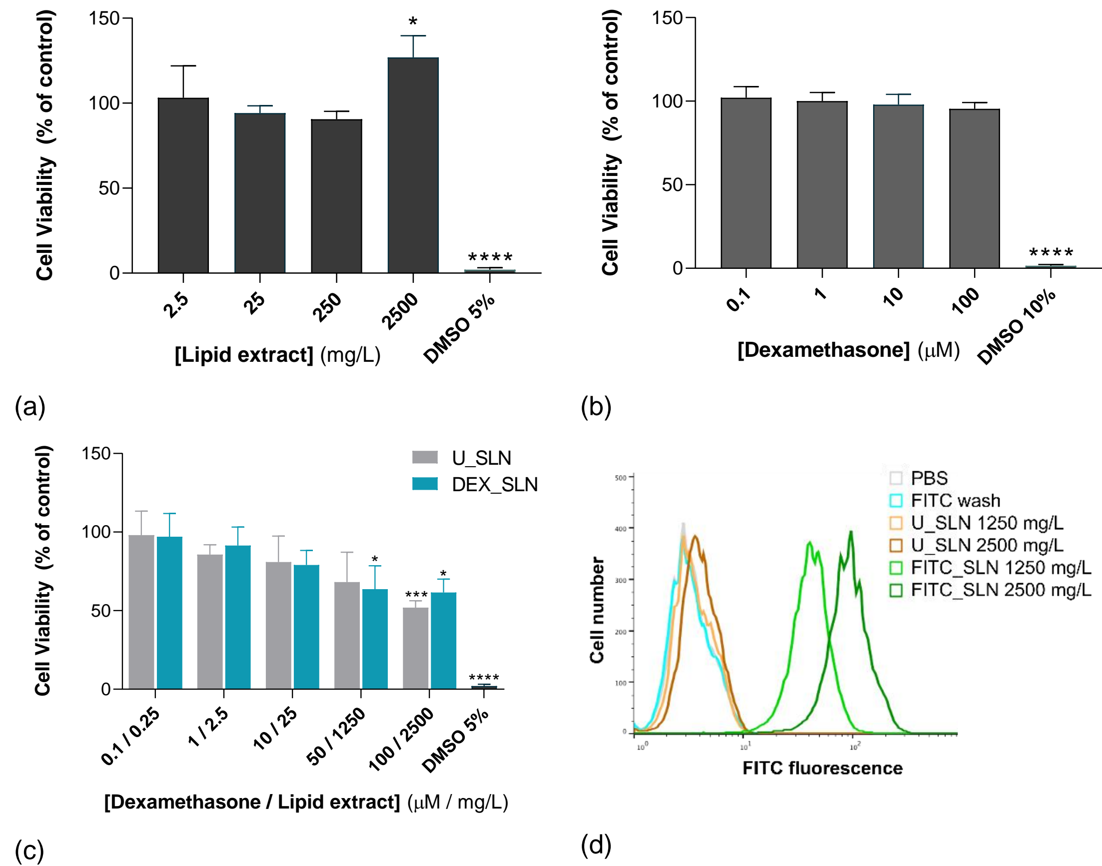

*Figure 3. Cell viability of HaCaT cells after treatment with (a) lipid extract; (b) dexamethasone; and (c) DEX-loaded and unloaded nanoparticles (DEX_SLN and U_SLN, respectively) for 24 h. Data from the MTT assay are expressed as percentages of the non-treated control cells using mean ± standard deviation (n=3). Significant differences compared to data obtained with the vehicle are identified with *, *** and **** (p-values < 0.05, 0.005 and 0.001, respectively). (d) Relative fluorescence of cells incubated with different concentrations of FITC-loaded or unloaded SLN. FITC dialysis wash was used as a control for nonencapsulated FITC internalization.*

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

4. Discussion

The present work aimed to probe the potential of the lipid extract from the biomass of BSF larvae as the core material of solid lipid nanoparticles with a dual function – efficient delivery of glucocorticoids and promotion of barrier function. The development strategy was based on a design of experiments (BBD) to obtain an optimized formulation capable of encapsulating a model drug (DEX), which was then assessed in terms of physicochemical characteristics, storage stability over time, drug release and cellular cytotoxicity and uptake.

Our data support that BBD is a powerful tool for nanocarriers optimization, as previously described (12, 13). The optimized DEX-loaded SLN had satisfactory physicochemical characteristics, namely PS < 200 nm, a uniform distribution of particle sizes (PDI < 0.3), a promising colloidal stability (ZP lower than -30 mV), and elevated EE and LC values (higher than 85% and 1.2%, respectively). According to the literature (14, 15), these properties are adequate for topical application, being promising for the treatment of atopic dermatitis.

It is noteworthy that SLN formulated with insect larvae oil exhibit physicochemical properties for skin delivery that are comparable to those of SLN prepared using conventional commercial solid lipids, such as Precirol ATO5® (PA5), Compritol® 888 ATO and Dynasan® 114/118 (16, 17). A drawback associated with these commercial lipids is the requirement for elevated melting temperatures during the production method, as exemplified by PA5 and Compritol® (ca. 80 °C). BSF oil enables SLN preparation at 50 °C, apart from being extracted from insect larvae biomass, thereby reducing the energy and production costs and enlarging the SLN versatility for thermosensitive compounds.

SLNs are often impaired by long-term instability in terms of size, due to particle aggregation, and EE/LC, due to drug expulsion from the lipid matrix (14, 18). However, in this work, good physical stability was observed for the nanoparticles at room temperature for 60 days. In general, all characteristics were maintained and only discrete variations in pH values were observed after 14 days of storage. However, these values remained in an acceptable range for skin application (19). This data shows, once again, potential for the use of insect larvae oil as an innovative ingredient in SLN-based topical formulations.

In order to evaluate the potential of the optimized formulation for AD management, the release of DEX from the lipid particles was investigated for 26 h. Approximately 60% of the drug was released within the first 4 h, reaching a plateau after 8 h and achieving an almost complete release at 15 h (97%). In a study with cationic polymeric nanocapsules, Beber et. al (20) observed a DEX release of around 45% after 5 h. Nonetheless, different results were observed when DEX was encapsulated in lipomers systems, where at 20 h only 60% of the drug had been released from the nanosystem (21). Although the incorporation of polymers may aid in delaying the release of DEX, insect larvae oil-based solid lipid nanoparticles (SLN) demonstrate a sufficiently controlled drug release profile, suitable for a daily application regimen.

In order to analyze the cytotoxicity of DEX, BSF lipid extract and DEX-loaded and unloaded SLN, an MTT assay was conducted with HaCaT cells. The drug, the lipid extract and the unloaded and drug loaded SLNs (up to 10 µM of drug) were found to be cytocompatible. Interestingly, wells treated with the highest concentration of the lipid extract seem to exhibit an increase in cell proliferation, indicating that this is a promising

https://doi.org/10.26434/chemrxiv-2024-3fw8h ORCID: https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. License: CC BY-NC-ND 4.0

biomaterial for future cutaneous formulations, even at high concentrations. The results from the cellular uptake assay yielded high internalization of SLN in concentrations up to 2500 mg/L of lipid extract. These data confirm the in vitro safety of these nanocarriers for skin applications and their availability to reach the intracellular target of glucocorticoids in keratinocytes.

Altogether, this proof-of-concept study reinforces that insect larvae oil is a promising biomaterial to formulate SLN for DEX delivery in the context of the management of atopic dermatitis. Ongoing investigations are delving into the in vivo safety of unloaded nanocarriers crafted from insect larvae oil, as well as their effectiveness in forming an occlusive film and reinstating skin barrier properties.

Funding: This research was funded by FCT - Foundation for Science and Technology, I.P. under DOI 10.54499/UIDP/04567/2020, DOI 10.54499/UIDB/04567/2020 and DOI 10.54499/EXPL/BTM-MAT/0112/2021 projects attributed to CBIOS, as well as for the research grants attributed to C.A. (UI/BD/151423/2021) and J.V.

Acknowledgments: The authors thank Entogreen® company for kindly providing the BSF larvae used in this study.

Conflicts of Interest: The authors declare no conflict of interest.

References:

1. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. Lancet 2020; 396(10247): 345-60. https://doi.org/10.1016/s0140-6736(20)31286-1.

2. Yakupu A, Aimaier R, Yuan B, Chen B, Cheng J, Zhao Y, et al. The burden of skin and subcutaneous diseases: findings from the global burden of disease study 2019. Front Public Health 2023; 11: 1145513. https://doi.org/10.3389/fpubh.2023.1145513.

3. Almeida C, Filipe P, Rosado C, Pereira-Leite C. Nanodelivery Strategies for Skin Diseases with Barrier Impairment: Focusing on Ceramides and Glucocorticoids. Nanomaterials 2022; 12(2): 275. https://doi.org/10.3390/nano12020275.

4. Danso M, Boiten W, van Drongelen V, Gmelig Meijling K, Gooris G, El Ghalbzouri A, et al. Altered expression of epidermal lipid bio-synthesis enzymes in atopic dermatitis skin is accompanied by changes in stratum corneum lipid composition. J Dermatol Sci 2017; 88(1): 57-66. https://doi.org/10.1016/j.jdermsci.2017.05.005.

5. Imokawa G. Cutting Edge of the Pathogenesis of Atopic Dermatitis: Sphingomyelin Deacylase, the Enzyme Involved in Its Ceramide Deficiency, Plays a Pivotal Role. Int J Mol Sci 2021; 22(4). https://doi.org/10.3390/ijms22041613.

6. Wollenberg A, Kinberger M, Arents B, Aszodi N, Avila Valle G, Barbarot S, et al. European guideline (EuroGuiDerm) on atopic eczema - part II: non-systemic treatments and treatment recommendations for special AE patient populations. J Eur Acad Dermatol Venereol 2022; 36(11): 1904-26. https://doi.org/10.1111/jdv.18429.

7. Wiedersberg S, Leopold CS, Guy RH. Bioavailability and bioequivalence of topical glucocorticoids. Eur J Pharm Biopharm 2008; 68(3): 453-66. https://doi.org/10.1016/j.ejpb.2007.08.007.

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

8. Souto EB, Fangueiro JF, Fernandes AR, Cano A, Sanchez-Lopez E, Garcia ML, et al. Physicochemical and biopharmaceutical aspects influencing skin permeation and role of SLN and NLC for skin drug delivery. Heliyon 2022; 8(2): e08938. https://doi.org/10.1016/j.heliyon.2022.e08938.

9. Almeida C, Rijo P, Rosado C. Bioactive Compounds from Hermetia Illucens Larvae as Natural Ingredients for Cosmetic Application. Biomolecules 2020; 10(7). https://doi.org/10.3390/biom10070976.

10. Almeida C, Murta D, Nunes R, Baby AR, Fernandes Â, Barros L, et al. Characterization of lipid extracts from the Hermetia illucens larvae and their bioactivities for potential use as pharmaceutical and cosmetic ingredients. Heliyon 2022; 8(5): e09455. https://doi.org/10.1016/j.heliyon.2022.e09455.

11. ICH. ICH Harmonised Tripartite Guideline. Stability testing of new drug substances and products: Q1A (R2). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2003.

12. Pinto RM, Monteiro C, Costa Lima SA, Casal S, Van Dijck P, Martins MCL, et al. N-Acetyl-l-cysteine-Loaded Nanosystems as a Promising Therapeutic Approach Toward the Eradication of Pseudomonas aeruginosa Biofilms. ACS Appl Mater Interfaces 2021; 13(36): 42329-43. https://doi.org/10.1021/acsami.1c05124.

13. Vieira J, Castelo J, Martins M, Saraiva N, Rosado C, Pereira-Leite C. Mixed Edge Activators in Ibuprofen-Loaded Transfersomes: An Innovative Optimization Strategy Using Box-Behnken Factorial Design. Pharmaceutics 2023; 15(4). https://doi.org/10.3390/pharmaceutics15041209.

14. Souto EB, Baldim I, Oliveira WP, Rao R, Yadav N, Gama FM, et al. SLN and NLC for topical, dermal, and transdermal drug delivery. Expert Opin Drug Deliv 2020; 17(3): 357-77. https://doi.org/10.1080/17425247.2020.1727883.

15. Sguizzato M, Esposito E, Cortesi R. Lipid-Based Nanosystems as a Tool to Overcome Skin Barrier. Int J Mol Sci 2021; 22(15). https://doi.org/10.3390/ijms22158319.

16. Das S, Ng WK, Kanaujia P, Kim S, Tan RB. Formulation design, preparation and physicochemical characterizations of solid lipid nanoparticles containing a hydrophobic drug: effects of process variables. Colloids Surf B Biointerfaces 2011; 88(1): 483-9. https://doi.org/10.1016/j.colsurfb.2011.07.036.

17. Ezzati Nazhad Dolatabadi J, Hamishehkar H, Eskandani M, Valizadeh H. Formulation, characterization and cytotoxicity studies of alendronate sodium-loaded solid lipid nanoparticles. Colloids Surf B Biointerfaces 2014; 117: 21-8. https://doi.org/10.1016/j.colsurfb.2014.01.055.

18. Gordillo-Galeano A, Mora-Huertas CE. Solid lipid nanoparticles and nanostructured lipid carriers: A review emphasizing on particle structure and drug release. Eur J Pharm Biopharm 2018; 133: 285-308. https://doi.org/10.1016/j.ejpb.2018.10.017.

19. Ali SM, Yosipovitch G. Skin pH: from basic science to basic skin care. Acta Derm Venereol 2013; 93(3): 261-7. https://doi.org/10.2340/00015555-1531.

20. Beber TC, de Andrade DF, Chaves Pdos S, Pohlmann AR, Guterres SS, Beck RC. Cationic Polymeric Nanocapsules as a Strategy to Target Dexamethasone to Viable Epidermis: Skin Penetration and Permeation Studies. J Nanosci Nanotechnol 2016; 16(2): 1331-8. https://doi.org/10.1166/jnn.2016.11670.

21. Pena-Rodríguez E, Lajarin-Reinares M, Mata-Ventosa A, Pérez-Torras S, Fernández-Campos F. Dexamethasone-Loaded Lipomers: Development, Characterization, and Skin Biodistribution Studies. Pharmaceutics 2021; 13(4). https://doi.org/10.3390/pharmaceutics13040533.

https://doi.org/10.26434/chemrxiv-2024-3fw8h **ORCID:** https://orcid.org/0000-0001-5407-3983 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0