https://doi.org/10.31925/farmacia.2024.2.15 **ORIGINAL ARTICLE**

PRE-FORMULATION, FORMULATION AND PILOT SCALE-UP STUDIES TO ESTABLISH THE QUALITATIVE AND QUANTITATIVE COMPOSITION OF AN INNOVATIVE NANOFORM DIETARY SUPPLEMENT FOR MENOPAUSAL THERAPY

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Abstract

The present study aimed to establish the theoretical qualitative and quantitative formula of a nanoparticle-based dietary supplement suitable for menopause therapy by integrating various herbal active ingredients – *e.g.* diosgenin – into a nano lipid matrix with vegetable oils. NLCs (nanostructured lipid carriers) consist of nanoparticles of standardised extracts that are present in oily form, making it challenging to incorporate them into a powder mixture. Therefore, there is a need to use modern excipients that, through their properties, absorb the oil from NLCs and convert them into powder without breaking the nanocapsules, thus providing favourable technological attributes for their processing into solid pharmaceutical forms (capsules, tablets), further allowing the active ingredients encapsulated in the nanolipid matrix, an optimal release in the body. Once the formulation of the final product had been determined, the process was scaled up to the pilot batch. The amount of dissolved diosgenin was 75.8% for tablets and 81.1% for capsules. The physico-chemical and microbiological characterization of the solid pharmaceutical forms demonstrated that the developed products containing NLCs comply with the quality requirements of the critical parameters for both pharmaceutical forms. The successful transformation of NLCs, encapsulating active plant principles, into a stable powder form to form a solid oral form represents a pivotal step in pharmaceutical technology.

Rezumat

Prezentul studiu a avut ca scop stabilirea formulei teoretice a unui supliment alimentar pe bază de nanoparticule adecvat pentru terapia menopauzei, prin integrarea diferitelor principii active din plante (de exemplu, diosgenina) într-o matrice nano lipidică cu uleiuri vegetale. NLC-urile (transportatori lipidici nanostructurați) sunt compuse din nanoparticule de extracte uleioase, ceea ce face dificilă încorporarea lor într-un amestec de pulbere. Prin urmare, este necesară utilizarea unor excipienți moderni care, prin proprietățile lor, să absoarbă uleiul din NLC-uri și să le transforme în pulbere fără a sparge nanocapsulele, oferind astfel atribute tehnologice favorabile pentru prelucrarea lor în forme farmaceutice solide (capsule, tablete), permițând în continuare ingredientelor active încapsulate în matricea nanolipidică, o eliberare optimă în organism. După ce a fost determinată formularea produsului final, procesul a fost scalat la lotul pilot. Cantitatea de diosgenină dizolvată a fost de 75,8% pentru tablete și 81,1% pentru capsule. Caracterizarea fizico-chimică și microbiologică a formelor farmaceutice solide a demonstrat că produsele dezvoltate care conțin NLC-uri respectă cerințele de calitate ale parametrilor critici pentru ambele forme farmaceutice. Transformarea cu succes a NLC-urilor, care încapsulează principii active din plante, într-o pulbere stabilă pentru a forma o formă farmaceutică solidă reprezintă un pas esențial în tehnologia farmaceutică.

Keywords: nano lipid matrix, scale-up, pre-formulation, menopausal, NLC, *Evening primrose* oil, diosgenin, *liquorice* extract

Introduction

Novel pharmaceutical formulations for drug delivery systems include nanostructured lipid carriers (NLCs), which are considered an alternative to already established solid lipid nanoparticles (SLNs) [1]. This new generation of SLNs improved the controlled release of poor hydrophilic drugs and possessed excellent physicochemical stability [2, 3] and environment-friendly behaviour [4, 5]. NLCs are bicomponent nanostructures containing solid and liquid lipids as a solid matrix [6, 7]. Thus,

a NLC matrix has the advantage of increasing a plant extract's bioavailability [8, 9].

Current research data provide insights into the exploratory efforts aimed at synthesizing oral solid forms of NLCs for enhancing the bioavailability of the active ingredient; oral NLCs are studied for being superior to other nanoforms due to better retaining the content and lower leakage of entrapped bioactive molecules, better regulation of the size and release process (Katouzian *et al*.; Pyo, Müller and Keck) [10, 11]. In certain studies, NLC's are candidates for enhancing the bioavailability of pharmacological entities in pharmaco-kinetic class IV BCS (Biopharmaceutical Classification System) that have poor aqueous solubility and permeability [12], such as the case of diselenide, an antiparasitic drug active in Leishmaniasis [13]. Also, in the field of nutraceuticals, NLCs are potential bioactivity enhancers of the food bioactive molecules with the same low solubility and low permeability profiles [14]; targeting of nanocapsules towards specific biochemical or cellular targets by specific bioactive ligands is also using inorganic mesoporous *silica* nano-encapsulation [15].

The oral NLC forms are studied for higher muco-adhesive properties, a modified interaction with the gastrointestinal enzymatic and acido-basic system, conferring a protective envelope for the labile or less bioavailable active ingredient, enhancing bioavailability, having better acid stability; however, the lipid nano-capsule showed des encapsulation property and release of the bioactive in alkaline bioidentical environment in special dissolution studies [16]; vulnerable to lipolytic pancreatic enzymes; formulating it with inert excipients may confer a protection barrier. The enzymatic break-down of NLCs can be a double-edged sword: on one hand, it can facilitate the release and absorption of the encapsulated drug, enhancing its bioavailability. Based on the "Trojan horse effect" [17], after intake of the triglycerides, lipid matrices of NLC are digested by pancreatic lipases into mono- and di-glycerides. In the presence of bile salts, the monoglycerides may form mixed micelles that still contain the bioactive substance. Afterward, these lipids may undergo absorption together with the drug *via* chylomicron formation primarily into the lymphatic system, which is confirmed with fluorescent tracers [18, 19]; the NLC carrier system overpasses the liver and the first pass effect [20]. The digestion of the lipid matrix by pancreatic enzymes releases the drug in a more soluble form, aiding its absorption in the intestine. (b) On the other hand, excessive or premature breakdown of the lipidic nano capsule could potentially reduce the protective benefits of NLCs, such as sustained release profile delivery of the active ingredient in a classical, not facilitated, way.

Few available therapies in menopause show an optimal therapeutic approach in the context of a physiological status characterised by an estro-progestative decline in a healthy woman [21, 22]. In the past decade, herbal and dietary supplements have gained use for different health problems [23], including menopause symptomatology, body weight control, depression and insomnia [11, 12]. Diosgenin is one of the substances used for controlling menopause or cognitive illness with a nontoxic nature proved in different trials [24, 25]; however, further clinical investigations are needed to propose a better-investigated safety profile. Less is known in the literature about the scaling-up process in the pilot plant during the NLC manufacturing phases and incorporation in solid oral forms. In previous research stages, we used wild yam extract standardised to diosgenin and transformed it into a nano product [26, 27]. Based on data from our previous studies [28], the purpose of the present study is (a) to incorporate the lipid matrix of NLCs - formulated in a previously published research [29] - into a powdery mixture suitable for compression and capsule filling in order to obtain a final pharmaceutical form of tablets/capsules and (b) to perform the technological transfer of the NLC solid oral forms selected in the pre formulation-formulation stages to the pilot batch size (the scale-up process).

Materials and Methods

We have developed the present study in three steps: (1) pre formulation of the lipidic nano capsules solid oral form (selection and control of the ingredients), (2) formulation of the NLC oral solid forms as tablets and capsules with the selection of the best formulas from the study plan, (3) production of the selected formulas in the previous stage at the pilot scale, validation and quality control of the final product (one tablet formula and one capsule formula) to enter the preclinical and clinical study in a following research stage.

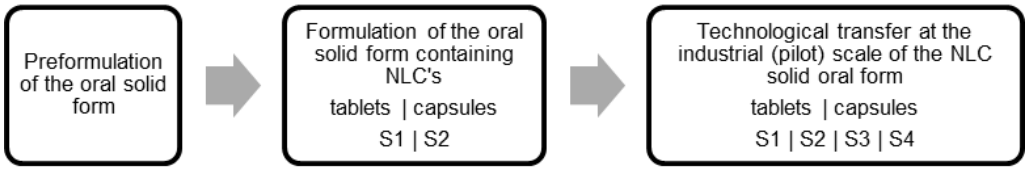

Figure 1.
Graphic representation of the study plan

Materials

Raw NLCs

NLC active components: SBO—soybean oil; (J.S. Hamilton, Gdynia, Poland); FSO—flaxseed oil (*Linus usitatissimus*, J.S. Hamilton, Gdynia, Poland); SMO—*Silybum marianum* oil, J.S. Hamilton, Gdynia, Poland); DSG—diosgenin (wild yam standardised extract) *Dioscorea opposita* Thunb. *radix* (*Cactus Botanics* 95% DSG CA, USA); GA—glycyrrhizic acid (liquorice standardised extract) (Organic Herb Inc., Changsa, China); TTG—triterpene glycosides (black cohosh standardised extract); (*Cimicifuga racemosa radix* 2,5% triterpene, Organic Herb Inc., Sanhai, China); PP—polyphenols (resveratrol extract) Benepure Pharmaceutical Co Ltd. Chengdu, China.

The NLCs taken into study contained active diosgenin/ glycyrrhizic acid principles in soybean or evening primrose oil and diosgenin/triterpene glycosides in flaxseed oil (S1, S2, S3, S4, the AC HELCOR R&D Centre, Baia-Mare, Romania & The Polytechnic University of Bucharest, Romania) (Table I and Figure 2).

Table I

Samples taken into study – short coding of the Raw NLC samples

No.	Code	NLC type	Pre-formulation stage	Formulation stage
1	**S1**	NLC-ULN-DSG-ELD1	yes	yes
2	**S2**	NLC-US-DSG-ELD	yes	yes
3	**S3**	NLC-ULN-DSG-ELD2	no	yes
4	**S4**	NLC-UIn-DSG-BCoh	no	yes

* ULN - *Evening Primrose* oil, DSG - Wild yam extract standardised to diosgenin, ELD - *Licorice* extract standardised to glycyrrhizin acid, US - *Soybean* oil; UIn - *Flaxseed* oil, BCoh - *Black Cohosh* extract standardised to triterpene glycosides;

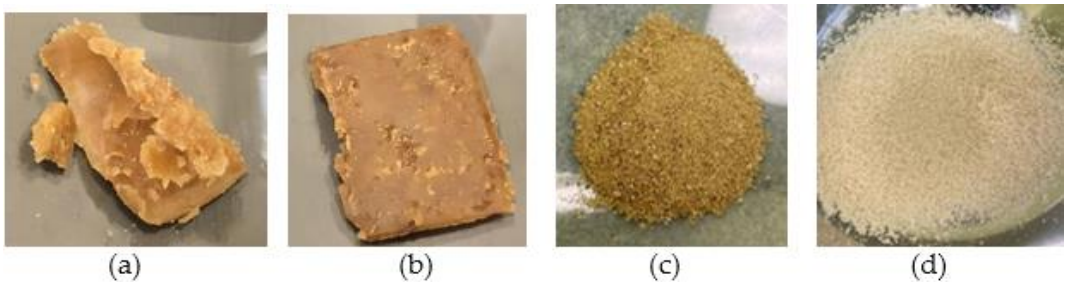

Figure 2.

NLC samples took into study: (a) NLC-S1; (b) NLC-S2 (c) NLC–S3; (d) NLC-S4

Excipients used in the formulation of oral solid form (tablets/capsules)

Sorbitol (NEOSORB P 60W, Roquette); *Silica* colloidal anhydrous (HDK N20 - Pyrogenic *Silica*, Wacker); Hydroxypropyl cellulose (KLUCEL, Ashland); Mannitol (PEARLITOL 160C, Roquette); Magnesium Stearate (Magnesium Stearate EP, Mosselman); Calcium Carbonate (Calcium Carbonate, AIS & A Prodimpex); Calcium Phosphate Anhydrous (DI-CAFOS A60, Budenheim); Microcrystalline Cellulose 102 (ACECEL 102, Sigachi Industries Limited); Magnesium Alumino-metasilicate (NEUSILIN UFL2, Fuji Chem. Ind.); Spray-dried granular amorphous *Silica* (FUJISIL, Fuji Chemical Industries).

Methods

Preparation of the raw NLCs for the pharmaceutical oral solid form

During the preparation of the raw NLCs to be ready for the pilot scale, we considered the following criteria: (1) the preparation should follow a coherent and reproducible manufacturing flow, (2) the process could be scaled-up (laboratory-pilot-industrial), (3) the product would present stability over time, not to be toxic and (4) the nanoformulation would improve biopharmaceutical performance and absorption of the active ingredient.

For obtaining the raw NLCs we followed the steps of: (1) weighing the raw materials (balance BIZERBA® GE 3000-23, Balingen, Germany) (2) homogenising under constant stirring using magnetic agitation on electric hobs (MS-H-Pro+ MQ216AV0002193, Beijing, China) the three main components - solid and liquid lipids phase, the aqueous phase containing surfactants and co-surfactants as, Tween 20, Span 80 (SPAN™ 80-LQ-MV, Croda Europe Ltd), Poloxamer 188 (Poloxamer 188 PRO, Sigma,-Aldrich), Phosphatidyl-choline (UNILEC® PDSL - Unilecithin) and the plant extracts by maintaining at 70 - 75°C to prevent solidification of lipid component; (3) homogenisation of the primary emulsion using a high-speed mixer - High Shear Homogenizer (OMNI MACRO HOMOGENISER® 17-1800-02, Kennesaw, Georgia); (4) reducing the size of the active principle to the nano level with the help of the High-Pressure Homogenizer (SPX APV 2000®, Bydgoszcz, Poland); (5) analysing the emulsion with the ZetaSizer equipment in terms of particle size, polydispersity and Zeta potential (Malvern® ZSU3200, Malvern, United Kingdom); (6) cooling the nano dispersions at room temperature, then mixed with 20% trehalose solution, stored overnight in a freezer (-16°C ~ -18°C) and (7) freeze-dried by lyophilization (-55°C, 0.05 mbar, 72 hours, using

Lyovapor L-300 Pro Freeze Dryer, (BÜCHI Labor-technik® AG, Germany).

Preparation of pharmaceutical oral solid form containing raw NLCs

Pre formulation of pharmaceutical oral solid form containing raw NLCs took into study.

Preliminary test on blank raw NLCs: We performed a preliminary test on blank oily NLCs combined with mannitol and trehalose to check if we can obtain a less-oily raw NLC and therefore more suitable for use in a pharmaceutical oral solid form formulation. Four (empty) control nano capsules (NLC-US) with no active principles, incorporating different percentages of cryoprotective agents were prepared and analysed for appearance. The T1-T2 samples were based on mannitol 5%, respectively 10% and the T3-T4 samples were based on trehalose 5%, respectively 10%.

Experimental Plan: The experimental plan for testing the raw NLCs' combination with different excipients is presented in Supplementary Table I.

In this stage, we mixed the raw NLCs S1 and S2, containing active substances, with the ten selected excipients and obtained different mixtures of the NLCs in order to select the proper excipient suitable for obtaining a pharmaceutical solid form.

The raw NLCs S1 and S2 were divided into 12 parts (ten to be tested according to the Experimental Plan and two as spare samples). Each of the 10 parts of each NLC sample was weighed (balance PRECISA® 350-8860/R, Dietikon, Switzerland) and incorporated manually together with the excipients.

We sieved the mixture (2 mm sieve); the excipient mass required for homogenization was calculated considering the target percentages of the excipient in the final formulation and the rate of NLC in the final formulation (30%)**:**

$$m = (a \times b)/30,$$

(m = excipient mass (g), a = NLC mass (g), b = target percentage of excipient in the final formulation)

We've analysed the following parameters: (1) appearance, (2) properties of excipients to absorb oil from NLC and (3) properties of excipients to form a powder mixture with NLC suitable for compression and/or encapsulation in order to establish the qualitative composition of the pharmaceutical oral solid form containing raw NLCs.

Formulation and optimization of the pharmaceutical oral solid form containing raw NLCs

We conditioned the NLC S1 and NLC S2 powder developed in the previous stage as oral solid forms, containing spray-dried granular amorphous *Silica* (FUJISIL, Fuji Chemical Industries) as a selected excipient because the volume of the capsule limits the volume of the filling mixture, we tested (a) capsules - for an average NLC powder mass of up to 220 mg NLC powder *per* capsule; (b) tablet - for an average NLC powder mass over 220 mg/tablet, reaching average mass values of 600 - 660 mg *per* tablet, testing both technological processes of formulating the finished form (both capsules and tablets).

In the technological process, we used the eccentric tablet machine Matrita® (Odorheiu-Secuiesc, Romania); the encapsulation was done using the capsule filling machine Capsule Connection® LLC (Arizona, USA).

Scale-up to pilot size

During the procedure for optimising the formulation and the manufacturing process, the following critical process parameters and quality attributes of the resulting product were followed: powder/granule flowability, mould filling, sticking to the equipment parts, the appearance of the product (uniform, homogeneous, intact edges), average mass and uniformity of mass, according to the European Pharmacopoeia (EPh) regulations [30].

The homogenization ratio between the NLC and the spray-dried granular amorphous *silica* is adapted according to the appearance of the NLC sample (how much oil must the excipient absorb to become a powdery mixture). Homogenization parameters: manual homogenization; time: 5 minutes/100 g sample. The mixture obtained had a homogeneous structure, and good flowing properties and finished products were obtained, but it showed a strong tendency to stick on the punches and dies wall surface. A lubricant must be added.

Compression tests were performed on two types of punches, d = 7 mm and d = 13 mm, to see if the sticking phenomenon is accentuated with the increase of the contact surface of the punch diameter, respectively. Compression parameters: compression force: 50 - 55 kN, compression speed: 30 tab/min.

Quality control methods for the pharmaceutical oral solid form

The quality parameters investigated for final products, appearance, colour, average mass, disintegration, microbiological tests and assay were assessed in compliance with European Pharmacopoeia guidelines [30] and dissolution was performed after an in-house method.

The test for microbiological quality was performed according to the relevant method described in the European Pharmacopoeia 9 - 2.6.12 and 2.6.13: (a) microbiological examination of non-sterile products (TAMC and TYMC), count plate method, (b) microbiological examination of non-sterile products (*E. coli*): test for specific micro-organisms, *Escherichia coli.* The microbiological parameters and their requirements are TAMC (Total aerobic microbial count): NMT 10^4 CFU, TYMC (Total yeast and molds count): NMT 10^2 CFU and *Escherichia Coli (per* 1 g*)* which must be absent. We have further performed the microbiological stability testing of the formulated NLCs solid oral form (tablets/capsules) three and six months after manufacturing, *per* the provisions of the European Pharmacopoeia 9 - 2.6.12 and 2.6.13.

The dissolution test was performed in the dissolution medium sodium lauryl sulphate - purified water 1.0 g/L, pH 6.9, using device 1 (USP), baskets – Hanson® SR8 PLUS at 100 rpm, with an average dissolving volume of 250 mL, at 37 ± 0.5°C, for 60 minutes. The filter used for sampling was PTFE type, with 0.45 micrometres pore size—reference substance: Diosgenin *r.s.*

Dissolution conditions: Apparatus: SR 8-PLUS (Hanson Research), equipped with baskets element; Dissolution medium: sodium lauryl sulphate 1.0 g/L; The volume of dissolution medium: 250 mL; Temperature: 37 ± 0.5°C; Rotation speed: 100 r.p.m.; Sampling time: 60 minutes. *Reagents:* acetonitrile HPLC; sodium lauryl sulphate 1 g/L: 1 g sodium lauryl sulphate is dissolved in water and diluted to 1000 mL with purified water; orthophosphoric acid 85%; purified water; *Chromatographic conditions:* Equipment: JACSO isocratic HPLC with UV detector, thermostat and degasser; Column: stainless steel 150 mm x 4.6 mm ID; Packaging: Mediterranean Sea, C18; 150 x 4.6 mm, particle size: 3 µm; Mobile phase: acetonitrile: purified water:orthophosphoric acid 85% (900:100:0.05) (v/v/v); the mixture is homogenised and degassed. Temperature of the column: 30°C; Flow rate: 1.0 mL/ min; Detection: UV, at 206 nm; Injection volume: 100 µL; Calculation mode: external standard method. *Method*: Transfer the dissolution medium (sodium lauryl sulphate 1 g/L) into the spherical bottomed glass beaker and adjust the temperature indicated. Place one capsule into the vessel and start stirring. After 60 minutes elapsed, stop the stirring and withdraw 50 mL of the sample to be tested from all six vessels and filter through a paper filter (white), discarding the first 10 mL then through 0.45 µm filter.

Preparation of Solutions – for determination of dissolved diosgenin in dissolution medium. Reference solution: 10 mg diosgenin r.s. is dissolved in ethanol and diluted to 100.0 mL with the same solvent in a volumetric flask; 1.5 mL of this solution is diluted to 10.0 mL with sodium lauryl sulphate 1 g/L (0.015 mg/mL diosgenin).

Measurement: Equilibrate the column with the mobile phase. Inject 100 µL of the reference solution and 100 µL of the test solution and record the chromatograms. Measure the areas of the main peaks in the two chromatograms and calculate the amount of diosgenin dissolved/ tablet or capsule, %.

Results and Discussion

Pre-formulation studies results

Preliminary test on blank raw NLCs results

We performed a preliminary test on blank oily NLCs combined with mannitol and trehalose to check if we could obtain a less-oily raw NLC and, therefore, more suitable for use in a pharmaceutical oral solid form formulation.

Four (empty) control NLCs: T1, T2, T3, T4, with no active principles, were lyophilised with different percentages of cryoprotective agents. The T1-T2 samples were based on mannitol 5%, respectively 10% and the T3-T4 samples were based on trehalose 5%, respectively 10%, presented in Table II.

Table II

Blank samples treated with mannitol and trehalose

1	**T1**	NLC-US (5% Mannitol)
2	**T2**	NLC-US (10% Mannitol)
3	**T3**	NLC-US (5% Trehalose)
4	**T4**	NLC-US (10% Trehalose)

The blank NLCs formulated with mannitol and trehalose in different concentrations were inappropriate for direct conversion in a free-flowing powder. However, the aspect of the T4 sample was improved, concluding that a 10% amount of trehalose in the lyophilization phase is beneficial in obtaining a solid form based on raw NLCs.

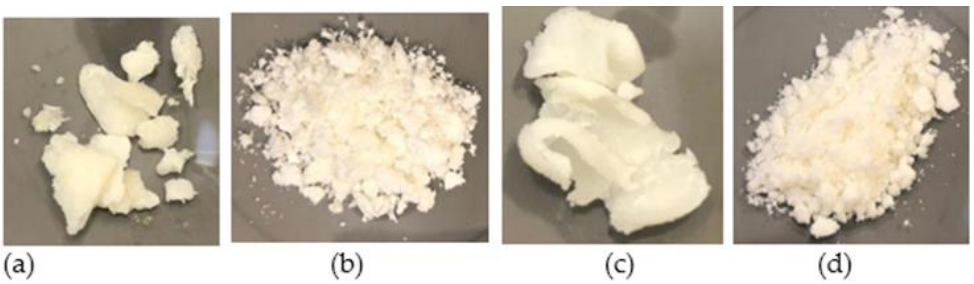

Figure 3.

(a) NLC-T1, (5% mannitol); (b) NLC-T2 (10% mannitol); (c) NLC-T3 (5% trehalose); (d) NLC-T4 (10% trehalose)

Experimental Plan Results

Based on literature data and technological specifications of excipients, we selected ten potential excipients to be tested in the pre-formulation phase with the tested samples in order to obtain a powder suitable for compression/encapsulation: sorbitol, *silica* colloidal anhydrous, hydroxypropyl cellulose, mannitol, magnesium stearate, calcium carbonate, calcium phosphate anhydrous, microcrystalline cellulose 102, magnesium alumino-metasilicate and spray-dried granular amorphous *silica*. Further on, we studied the capacity of these excipients, in different proportions of homogenization with sample

S1 and sample S2, to absorb the fatty base from the NLC structure and facilitate obtaining a powdery mixture suitable for tablet or encapsulation.

The results of NLC S1 sample homogenizations with the excipients selected according to the Experimental Plan are summarised in Supplementary Table II and Supplementary Figure 1.

The results of NLC S2 sample homogenization with the excipients selected according to the Experimental Plan are summarised in Supplementary Table III and Supplementary Figure 2.

During the experimental process, we have observed that sorbitol, hydroxypropyl cellulose, mannitol, magnesium stearate, calcium carbonate, calcium phosphate, microcrystalline cellulose do not have good absorption properties and the resulting mixture presents itself as a sticky mass, unsuitable for further processing, sorbitol, hydroxypropyl cellulose, mannitol, magnesium stearate, calcium carbonate, calcium phosphate, microcrystalline cellulose.

When we took into study the anhydrous *silica* colloidal at a proportion of 0.25 - 1% we had results showing that it does not have good absorption capacity. At proportions of 5% and 10% respectively, the absorption capacity of the fatty phases improved significantly, the appearance of the resulting mixture being an appropriate one. However, because the recommended amount is 0.5 - 3%, *silica* colloidal anhydrous was not chosen as an excipient to continue the study.

Magnesium alumino-metasilicates are recommended to be used in a proportion of 10 - 50%, respectively spray-dried granular amorphous *silica* recommended to be used in proportion of 10 - 30%, both have good absorption properties and the appearance of the resulting mixture being appropriate. In Figure 4 and Figure 5 we illustrate the successful results obtained at this stage for sample S1.

Figure 4.
Homogenization mixtures - S1 and magnesium alumino-metasilicate in various percentages: (a) S1.28; (b) S1.29; (c) S1.30; (d) S1.31

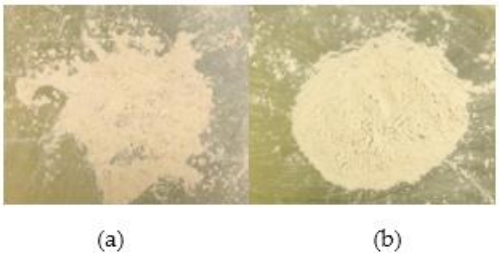

Figure 5.
Homogenization mixtures - S1 and spray-dried granular amorphous *silica* in various percentages: (a) S1.32; (b) S1.33

In Figure 6. and 7. we illustrate the successful results obtained at this stage for sample S2.

When we tested the capacity of oil absorption, the mixture aspect was according to the desired consistency and aspect. We have tested the flowability using a powder flow tester - ERWEKA® GmbH, with a 10 mm nozzle and a stainless steel flat bottom with a concentric circular orifice, end height diameter H = 4 mm and calculated the average value from the mass flow rate at 100 g of mixture; we have obtained 3.5% - 4.8% mass flow rates Q (g/s). We have tested the compressibility; the mixture could be compressed using a compression force of 50 - 55 kN at the tableting machine, AU type.

Figure 6.
Homogenization mixtures - S2 and magnesium alumino-metasilicate in various percentages: (a) S2.28; (b) S2.29; (c) S2.30; (d) S2.31

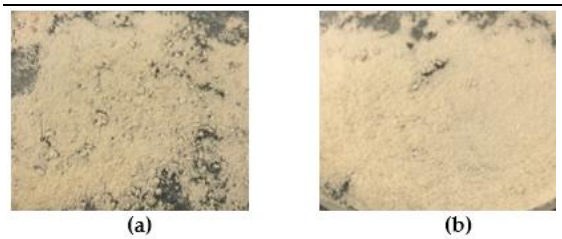

Figure 7.
Homogenization mixtures – S2 and spray-dried granular amorphous *silica* in various percentages: (a) S2.32; (b) S2.33

The measurements were performed at a controlled ambient temperature (21 ± 1°C) and relative air humidity of 50 ± 10%. The granular amorphous *silica* and the magnesium alumino-metasilicate were further taken into study as they showed the best parameters in the formulation of a capsule/tablet containing lipid nanostructure encapsulated active principles (NLC). Taking into account the results above, two of the ten excipients taken into the pre-formulation study showed favourable profiles: (1) the spray-dried granular amorphous *silica* and (2) the magnesium alumino-metasilicate (Figure 8), but the spray-dried granular amorphous *silica* was chosen as excipient for next step in terms of: (a) capacity of oil absorption (b) flowability of mixture (c) compressibility.

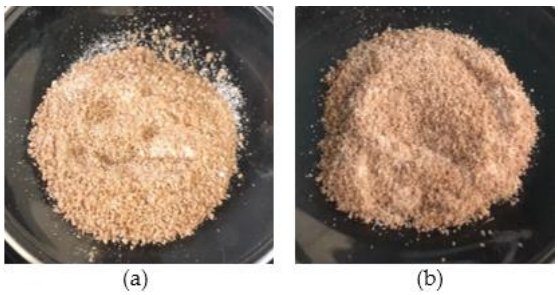

Figure 8.
Example of a homogenised mixture of NLC and (a) spray-dried granular amorphous *silica* (*silica* colloidal hydrated) (b) magnesium alumino-metasilicate

Formulation and optimization of the finished product results

The four studied samples in the formulation stage (S1, S2, S3, S4) differ by: (1) the extraction oil used, *Evening primrose* oil for samples S1 and S3, *soybean* oil for sample S2 and flaxseed oil for sample S4, respectively; (2) all four samples contain diosgenin, but S1, S2 and S3 also contain liquorice extract and S4 *black cohosh* extract; (3) samples S1 and S2 were not freeze-dried with a cryoprotective agent; therefore, their texture is very oily and sticky; (4) samples S3 and S4 were freeze-dried in the presence of trehalose; therefore, their appearance improved - not so oily and can be processed efficiently in a solid pharmaceutical form.

Tests have confirmed that the tendency to stick increases as the diameter of the punches increases (Figure 9 and Figure 10); the tablets were not suitable as the edges were not intact – chipping/sticking and upper and lower segments of the tablets separated horizontally – capping of the tablets.

Figure 9.
Tablets resulted from *NLC: spray-dried granular amorphous silica* powder mixture on d = 7 mm punches

Figure 10.
Tablets resulted from *NLC: spray-dried granular amorphous silica* powder mixture on d = 13 mm punches: capping and lamination

An anti-adherent agent was further added; we chose as lubricant ~1% magnesium stearate. It was a real challenge to obtain - from an amorphous, sticky substance with the appearance of a solid paste - a slightly granular powder with good flow properties, compressibility, non-adherence to the compression or capsule-filling equipment parts and that would confer the specific attributes of the pharmaceutical form, especially assay of the active ingredient and dissolution.

After homogenization of the selected NLC samples in the appropriate ratios with the main excipient (spray-dried granular amorphous *silica* with the role of oil absorbent) and after adding an anti-adherent agent/lubricant (magnesium stearate), a mixture of powders with a uniform appearance, with good rheological properties was obtained. At this point, the residual moisture content of the obtained powder was analysed using Scaltec Moisture Analyzer SMO 01 (Germany), the result being 0.9 - 2.0%, meaning that the moisture content will not affect stability and compressibility.

The quantitative composition of the finished product, as a result of the formulation and optimization stage, was determined for hard gelatine capsules and for tablets.

We further processed the powder by (1) compression (single punch tablet machine with d = 13 mm, eccentric tablet press, using a compression force of 55 kN) (Figure 11), respectively, (2) encapsulation using

hard, cylindrical gelatine capsules with hemispherical ends, uniform on the surface, transparent, size "0" using the capsule filler device (Figure 12).

The physicochemical analysis of the finished products concluded that we obtained (a) round biconvex-shaped uncoated tablets with uniform appearance, compact and homogeneous structure, intact edges, d = 13 mm and (b) suitable hard, cylindrical gelatine capsules with hemispherical ends, uniform on the surface, transparent, size "0".

Figure 11.
Composition of finished product in the form of tablets

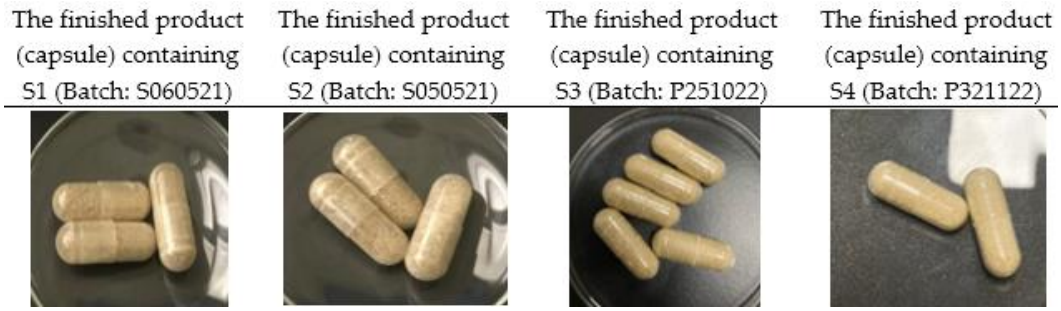

Figure 12.
The finished product in the form of hard gelatine capsules

Process scale-up results

Based on (1) Preliminary test on blank raw NLCs results and (2) Formulation and optimization of the finished product results, we selected NLC sample S3 as the best formulation obtained at the laboratory level for the technological transfer of the NLC through the manufacturing process; S3 consists of: (1) lipid phase (66%) – cetyl palmitate, glyceryl monostearate and *Evening primrose* vegetable oil; (2) aqueous phase (17%) – tween, poloxamer and phosphatidylcholine; (3) plant extracts (17%) – wild yam standardised to 95% diosgenin and liquorice standardised to 10% glycyrrhizic acid – with beneficial effects on menopausal states; (4) trehalose as a cryoprotective agent in the freeze-drying phase.

For the technological transfer validation, three raw NLC batches with the same formula (S3) and in the same size as the series used at the laboratory level were manufactured, but with different equipment (L1, L2, L3); for the transition from the laboratory scale to the pilot scale, another three batches were manufactured with the S3 formula but with batch sizes five times larger at the pilot level compared to the laboratory level (P1, P2, P3).

The NLC scale-up parameters and results are presented in Table III.

Corresponding results for the six studied batches analysed in triplicate were obtained, the size of the particles measuring between 107 - 163 nm, with a polydispersity indices between 0.168 and 0.2396.

Quality control of the pilot batch selected nanoforms

After successful scale-up and lyophilization of the pilot batch NLC emulsions, we manufactured the pilot batch finished products by (1) compression and (2) encapsulation using the excipients in quantities established in the pre-formulation, formulation and optimization studies.

The pilot batch formulation led to the production of round, lenticular light yellow tablets with uniform appearance, compact and homogeneous structure, intact edges and d = 13 mm deviation not exceeding ± 1%, respectively hard, cylindrical gelatine capsules with hemispherical ends, uniform on the surface, transparent, size "0".

The average mass was in accordance with limits stipulated by the European Pharmacopoeia (± 5%) compared to the ideal mass 660.00 mg for tablets and 220.00 mg of the contents of the capsule, which proves that the formulation has a good flow that results in uniform filling of the mould. The disintegration time showed ideal values for immediate-release pharmaceutical oral dosage forms, tablets or capsules within the permissible limit of a maximum of 15 minutes,

reinforcing the idea that the selection of types and quantities of excipients was correct. For all analysed tablets and capsules, diosgenin assay were obtained within the limits provided by the European Pharmacopoeia of ± 5% compared to the theoretical amount of 16.5 mg *per* tablet and 5.5 mg *per* capsule, proving a good homogeneity of the powder mixture. The amount of diosgenin dissolved after 60 minutes was within 70% for both pharmaceutical products and the microbiological tests were within the limits imposed by the European Pharmacopoeia. Storage conditions of the samples were: 2 - 8°C (refrigerated), 40 - 50% RH.

Table III

NLC Process scale-up parameters and results

Scale	**Laboratory**			**Pilot**		
Batch no.	L1	L2	L3	P1	P2	P3
Batch size	100 g (X)	100 g (X)	100 g (X)	500 g (5X)	500 g (5X)	500 g (5X)
Process parameters						
High Shear Homogenization	1 min/2000 rpm	1 min/2000 rpm	1 min/2000 rpm	1 min/4000 rpm	1 min/4000 rpm	1 min/4000 rpm
High Pressure Homogenization	3.2 min/500 bar	3.2 min/500 bar	3.2 min/500 bar	9 min/500 bar	9 min/500 bar	9 min/500 bar
Zeta Analyzer Results						
Z-Average, nm Specification: 0 - 1000 nm	107.1	119.2	162.7	157.7	154.3	139.9
Standard Deviation	0.5442	0.6489	0.8259	2.875	1.412	0.7205
RSD	0.5083	0.5442	0.5076	1.823	0.9152	0.5148
Zeta Potential, mV Specification: NA	-23.45	-23.99	-21.62	-19.66	-24.75	-20.21
Standard Deviation	3.522	0.1625	1.11	1.055	1.501	0.3256
RSD	15.02	0.6773	5.133	5.369	6.066	1.611
Polydispersity index Specification: < 0.3	0.168	0.1847	0.2215	0.2396	0.1574	0.2185
Standard Deviation	0.009739	0.004964	0.06155	0.03515	0.01848	0.0273
RSD	5.798	2.688	27.79	14.67	11.75	12.49
Populations Specification: pop. 1; NLT 95%	1: 99.26%: 126 nm 2: 1.105%: 2568 nm	1: 100%	1: 100%	1: 99.21%: 175 nm 2: 2.364%: 4727 nm	1: 100%	1: 100%
Conclusion	**Conforms**	**Conforms**	**Conforms**	**Conforms**	**Conforms**	**Conforms**

The microbiological examinations of non-sterile products (TAMC and TYMC): count plate method and micro-biological examination of non-sterile products (*Escherichia coli*): test for specific micro-organisms, *Escherichia coli*, showed no changes at the initiation of the stability study, after three months and at six months; the analysed samples have good microbiological stability over six months; they do not degrade and the results are consistent and within the stipulated admissibility limits.

The TAMC parameter for S2 had the highest value of 0.3 x 10^4 CFU, 3% of the maximum allowed of 10^4 CFU; TYMC and *E. coli* were absent throughout the analysed period. The TAMC parameter for S3 had the highest value of 0.4 x 10^4 CFU, which is 4% of the maximum allowed of 10^4 CFU and TYMC and *E. coli* were absent throughout the analysed period. The TAMC parameter of S4 had the highest value at 0.01 x 10^4 CFU, meaning 0.1% of the maximum allowed of 10^4 CFU; TYMC and *E. coli* were absent throughout the analysed period.

The QC results for the tablets and capsules containing NLC that encapsulates active principle diosgenin in nanoform are according to the specifications of the targeted quality parameters (appearance, colour, average mass, mg, disintegration, minutes, assay – mg diosgenin *per* tablet, dissolution %, - after 60 minutes, micro-biological test: TAMC – UFC/g, TYMC – CFU/g, *Escherichia coli per* g).

The selection of finished oral form (tablet and capsule) excipients for this study was based on their main attribute of absorbing the oil from the NLCs to find the ideal excipient to solve the formulation issues when dealing with sticky/oily nanostructured lipid carriers. The ten excipients selected following the compatibility studies were sorbitol, *silica* colloidal anhydrous, hydroxypropyl cellulose, mannitol, magnesium stearate, calcium carbonate, anhydrous calcium phosphate,

microcrystalline cellulose 102, magnesium alumino-metasilicate and spray-dried granular amorphous *silica*.
We established the experimental plan taking into consideration the literature data and our experience; sorbitol, mannitol, calcium carbonate, calcium phosphate anhydrous, microcrystalline cellulose 102 and magnesium aluminium-metasilicate are diluents with good flow, disintegration and compressibility properties [31, 32]. Furthermore, magnesium alumino-metasilicate serves a multitude of purposes when compared to other silicates. It possesses a vast specific surface area, one of its key features being high oil and water adsorption capacity.
Spray-dried granular amorphous *silica* (*silica* colloidal hydrated) has a unique internal structure and remarkable flow properties, giving high internal porosity and superior compressibility properties; it acts as a transporter and adsorbent of oils (up to 3.3 mL/g), while it remains as a free-flowing powder to assimilate them into solid pharmaceutical forms. *Silica* colloidal anhydrous has a small particle size and large specific surface area that gives it desirable flow characteristics. It is also used as an adsorbent dispersing agent for liquids in powders.
Hydroxypropyl cellulose, a binder with excellent compactness and adsorbent properties of NLC oils, has proven performance with a host of herbal and nutraceutical actives with no varying properties. Magnesium stearate was used for its anti-adherent role, known as a "flow agent", as it helps speed the manufacturing process by preventing ingredients from sticking to mechanical equipment. Microcrystalline cellulose 102 is a crystalline powder composed of porous particles and is used for its adsorbent, anti-adherent and diluent role.
By examining the results of pre-formulation tests with the nano lipid matrix with *Evening primrose* oil, diosgenin and liquorice extract (S1) and the nano lipid matrix with soybean oil, diosgenin and liquorice extract (S2), we obtained appropriate results (transforming an oily structure in powder form) with spray-dried granular amorphous *silica* and magnesium alumino-metasilicate excipients, which were selected, as the leading candidates in the formulation of a capsule or tablet pharmaceutical form.
No different behaviour of the two samples was found when homogenizing with the same type of excipient in the same amount; as such, it can be concluded that the extraction oil, soybean or *Evening primrose*, does not influence the process of obtaining the powder by homogenizing the NLC with selected excipients. By manufacturing different formulations of NLCs, we concluded that freeze-drying in the presence of trehalose, a cryoprotective agent, significantly improves the appearance of the lipid nanocarriers – they are less oily so that they can be processed more efficiently in a solid pharmaceutical form.
After successfully transforming the oily nanostructured lipid matrix, which encapsulates active principles from plant extracts, into a powder mixture, appropriate solid pharmaceutical forms were obtained by compression/ encapsulation, preserving the intact nanocapsules. Depending on the amount of active principle in a dosage form that is needed for administration, we can choose a tablet (for a larger therapeutical dose) or a capsule (for a smaller therapeutical dose). In this study, the manufacturing process and quality results were compliant for both forms.
Following the good NLC manufacturing scale-up process and technological transfer from laboratory batches to pilot batches, measurements with the DLS (Dynamic light scattering) method showed that the applied technique obtained particle sizes with an average of 100 - 200 nm.
For better stability and longer shelf life; optionally, the tablets can be covered with a special coating, which prevents their decomposition in the stomach, for a controlled release of the active ingredient (*e.g.*, for intestinal absorption); unlike capsules, tablets have the advantage of being more mechanically resistant and easier to handle in the packaging and distribution stages**.**
The solid pharmaceutical finished products containing nanoforms of active plant principles – diosgenin – manufactured after the pre-formulation and formulation studies presented above were assessed by physico-chemical analysis critical parameters. All tested parameters complied with the requirements. Diosgenin content/pharmaceutical form was within limits, proving that the manufacturing process (from obtaining the NLCs to the finished products in the form of tablets/ capsules) is well-controlled, reliable and reproducible. Dissolution results were above the imposed limit of 70%, and this quality attribute was reached regarding the release of active principles, respectively, *in vitro* testing.

Conclusions

This process illustrates a thorough and effective innovative approach and process for transforming lipid-based nanocarriers into solid pharmaceutical forms for obtaining tablets or capsules with nano-structured lipid carriers, ensuring the end product's quality, consistency and effectiveness. The described pre-formulation and formulation studies were monitored according to quality requirements, including active ingredient assay, dissolution, microbiological quality and stability.
Our study's findings demonstrate significant advancements in the formulation and development of solid pharmaceutical forms using nano lipid "carriers" (NLCs). The successful transformation of NLCs encapsulating active plant principles into a stable powder form represents a pivotal step in pharmaceutical technology.

Conflict of interest

The authors declare no conflict of interest.

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