COMPARATIVE DISSOLUTION STUDY OF A SOLID PHARMACEUTICAL FORM CONTAINING NANOSTRUCTURED LIPID CARRIER (NLC) INCORPORATING DIOSGENIN – CONVENTIONAL *VERSUS* BIORELEVANT DISSOLUTION MEDIA

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Abstract

The present study aims to test the *in vitro* bioavailability of diosgenin (DSG) from a solid finished pharmaceutical form containing nanostructured lipid carrier (NLC) that incorporates wild yam (*Dioscorea villosa* L.) standardized plant extract. The dissolution study was performed in (a) conventional and (b) special dissolution media - that closely reproduce the stomach and intestinal fluids composition. DSG has revealed a high potential of bioactivities, a growing interest in various disorders - cancer, hypercholesterolemia, menopausal disorders, inflammation, and several types of infections, but with low aqueous solubility and enhanced hydrophobicity. The role of the NLC formulation is to increase the bioavailability of DSG, by transforming it into a nanoproduct. The *in vitro* dissolution test is the most critical for evaluating the performance of a pharmaceutical product, chosen as a defining tool in characterizing the samples in this study. Suitable conventional media were applied for the dissolution method as a routine test in the quality control of the finished product, while we have chosen biorelevant media for further testing to modulate the formulation and posology of a candidate final formula for the best *in vivo* bioavailability and for a better understanding of the *in vivo* dissolution behaviour of the dosage form containing nano encapsulated diosgenin. The recommended pharmaceutical form for products containing diosgenin encapsulated in a nano lipidic structure is gastro-resistant; the optimal absorption of the active principle takes place at the upper intestinal level.

Rezumat

Prezentul studiu își propune să testeze biodisponibilitatea *in vitro* a diosgeninei (DSG) dintr-o formă farmaceutică solidă finită care conține purtător de lipide nanostructurate (NLC) care încorporează extract standardizat de plante de yam sălbatic (*Dioscorea villosa* L.). Studiul de dizolvare a fost efectuat în (a) medii convenționale și (b) speciale de dizolvare - care reproduc îndeaproape compoziția stomacului și a fluidelor intestinale. DSG a dezvăluit un potențial ridicat de bioactivități, un interes tot mai mare pentru diverse tulburări - cancer, hipercolesterolemie, tulburări de menopauză, inflamație și mai multe tipuri de infecții, dar cu solubilitate apoasă scăzută și hidrofobie îmbunătățită. Rolul formulării NLC este de a crește biodisponibilitatea DSG, prin transformarea acestuia într-un nanoprodus. Testul de dizolvare *in vitro* este cel mai critic instrument pentru evaluarea performanței unui produs farmaceutic, ales ca definitoriu în caracterizarea probelor din acest studiu. Medii convenționale adecvate au fost aplicate pentru metoda de dizolvare ca test de rutină în controlul calității produsului finit, în timp ce am ales medii biorelevante pentru teste suplimentare pentru a modula formularea și posologia unei formule finale candidate pentru cea mai bună biodisponibilitate *in vivo* și pentru o mai bună înțelegere a comportamentului de dizolvare *in vivo* al formei de dozare care conține diosgenină nanoîncapsulată. Forma farmaceutică recomandată pentru produsele care conțin diosgenină încapsulată într-o structură nanolipidică este gastrorezistentă; absorbția optimă a principiului activ are loc la nivelul intestinal superior.

Keywords: nanostructured lipid carriers; diosgenin; dissolution; bioavailability; gastric fluid; intestinal fluid

Introduction

The nanostructured lipid carriers (NLCs) are stable colloidal formulations with enhanced drug delivery profiles. Due to their physicochemical stability, bio-compatibility, biodegradability and controlled release of drug substances, NLC's have received increased attention in recent years [1, 2]. NLCs are modified solid lipid nanoparticles (SLNs), composed of liquid lipids mixed with a solid lipid to form a matrix of nanostructured solid particles [3, 4].

Called "the mother of hormones" and "medicinal gold", diosgenin ($C_{27}H_{42}O_3$, [(3β,25R)-spirost-5-en3-ol], DSG) is a steroidal sapogenin, aglycon of dioscin, derived from vegetables, used in the industrial synthesis of most of the therapeutically useful steroidal drugs [5]. DSG occurs abundantly in the dried rhizome of *Dioscorea villosa* (wild yam) - known as wild yam, colic, rheumatism root, devil's bones, four-leaf yam (cultivated in limited areas such as the Caribbean, West Africa and Polynesia) - *Dioscorea alata*, *Dioscorea nipponica*, *Smilax china*, *Dioscorea zingiberensis* C.H. Wright (DZW) and *Trigonella foenum graecum*. Naturally, DSG exists as the saponin forms (dioscin, gracilin and water-soluble saponins) where the aglycone is linked with glucose or rhamnose or both by a 3–C–O glucosidic bond (Figure 1).
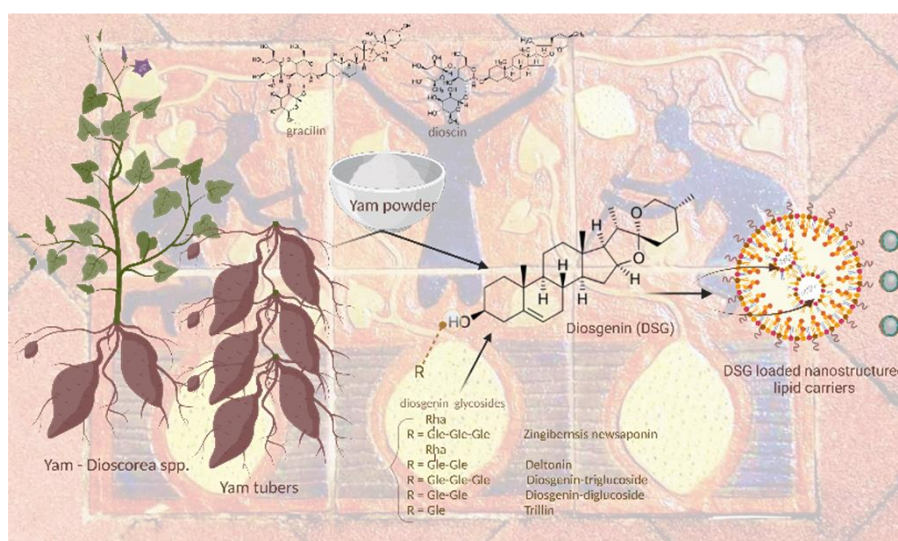

Figure 1.
Graphical representation of the extraction and synthesis of DSG-NLC [6, 7]

DSG is derived by acidic hydrolyzation of total steroid saponins [8]. Raw diosgenin is a white needle crystal or light amorphous powder with proven thermal and chemical stability, under various physical conditions has a relative molecular mass of 414.62; it is relatively stable against temperature and light exposure, strongly hydrophobic (LogP = 5.7), practically water-insoluble (0.7 ng/mL) and poorly soluble in other media (pH 1.2, pH 4.5, pH 6.8 and pH 7.4) [9]. However, it is highly soluble in most nonpolar organic solvents (such as chloroform, dichloroethane, propanol, ethyl acetate and propyl acetate) and partially polar solvents (such as acetone, methanol, and anhydrous ethanol) [10, 11]. DSG bioactive phytochemical is known to possess anti-inflammatory and antioxidant [12] properties and can be helpful, for instance, in blood and cerebral disorders [13, 14], allergic diseases, diabetes [15] and obesity [16, 17], menopausal symptoms [18, 19] and skin aging; it can also have a protective role in cardiovascular diseases (such as thrombosis and atherosclerosis) [20, 21] nephroprotection [22, 23], immunomodulator [24, 25] and antitumor activity [26, 27]. However, due to low solubility and reduced gastrointestinal absorption, DSG bio-disponibility is reduced [28]; the incorporation of an insoluble lipid matrix increases the bioavailability of a plant extract, wild yam extract - standardized to DSG, by transforming it into a nano product [29].

The reduced particle size of the NLC formulations improves the surface area of the NLC and allows efficient uptake in the intestine, particularly in the lymphoid section of the tissue - thus bypassing the first-pass metabolism. Another factor that facilitates the absorption of NLC in the intestinal milieu is its' high dispersibility. Besides this, the NLC can also adhere to the gut wall prolonging the residence time and, consequently, the absorption [30].

Dissolution is the process in which a substance forms a solution and measures the extent and rate of solution formation from a dosage form [31, 32]. The dissolution of a drug is essential for its bioavailability and therapeutic efficacy [33, 34]. Because dissolution is the most critical *in vitro* test for evaluating the performance of a pharmaceutical product, the dissolution test was chosen as the physicochemical testing method, being a defining tool in characterizing a sample. In addition,

the biorelevant dissolution media are used as a research tool for *in vitro - in vivo* correlation studies, modelling *in vivo* dissolution behaviour of immediate-release (IR) oral dosage forms [35, 36].

The main objectives of the study were (1) to determine the optimal absorption site of diosgenin encapsulated in a nano lipid structure (gastric environment, intestinal or colonic mucosa) and the moment: before or after the meal - using as tool the dissolution in special media that reproduce the real conditions in the human body; (2) to establish the routine dissolution analyses (QC control) of diosgenin encapsulated in a nano lipid structure, which are the recommended conventional dissolution media and which have a pH closer to that of the special media in which consistent results were obtained. We mention that conventional dissolution media is recommended for routine analyses due to the economic factor, which is much more advantageous in terms of cost.

To see if the excipients and the capsule in the finished product influence the release mode of diosgenin encapsulated in a nano lipid structure, standardized extract of wild yam - raw material, NLC - as an intermediate product containing nanostructured diosgenin and finished product containing NLC were tested.

Fasted State Simulated Gastric Fluid (FaSSGF), Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) are dissolution media that simulate human gut fluids. They contain physiological surfactants (bile salts and lecithin) present in the gastrointestinal tract to simulate these fluids much more accurately than conventional dissolution media. Fed Gastric Media (FEDGAS) dissolution media simulate human stomach fluids in the fed state. It contains fats and carbohydrates in a partially digested, high-fat FDA meal. Three different pH values (3, 4.5 and 6) reflect various stages of stomach emptying. Dissolution tests in FEDGAS are useful because they can help understand how the drug is released in the stomach after eating a meal.

Human fasted state colonic fluid (FaSSCoF) assesses how modified release drug products intended for colonic delivery dissolve in fluids in the fasted colon, while Human fed state colonic fluid (FeSSCoF) estimates how modified release drug products intended for colonic delivery dissolve in fluids in the fed colon. They contain the appropriate physiological components (bile salt and lipids) reflecting those present in colonic fluids before or after a meal. In general, they are less useful for immediate-release drugs because the absorption of most immediate-release drugs is mainly complete when the colon is reached, and the zone for absorption after oral administration is much smaller than the upper gastrointestinal tract.

To our best knowledge, this is the first dissolution study investigating DSG-loaded lipidic nano-capsules - performed in biorelevant media. The dissolution study was carried out on (1) wild yam plant extract, standardized to DSG (active principle), (2) on NLC containing this plant extract and (3) on a finished product formulated as a capsule containing the NLC as mentioned above, candidate for a finished product formula.

The study was performed in different dissolution media, to assess the comparative bioavailability data and to find the most suitable media for the dissolution of DSG for the (a) primary objective: selection of the appropriate dissolution medium to determine the bioavailability of diosgenin using conventional *in vitro* dissolution media - on the raw standardised extract, raw nanocapsules and the final form of oral finished product (gelatine capsules filled with the nanoproduct) - to set up the lab quality-control standards; (b) secondary objective: development of the analysis method that will be used later in the batch analysis of the finished product; (c) determination of the dissolution mode in biorelevant media, focused on the absorption place of the active principles from the chosen finished product, by simulating the *in vivo* conditions, similar to gastric, intestinal and colonic fluids, both in the fed and fasted state "stomach/ intestine/colon - full/empty" – to modulate the formulation and posology of the finished product for the best *in vivo* bioavailability.

Materials and Methods

Study Plan

The present study investigated the dissolution of DSG from various test samples in conventional media (acidic solutions, buffers, surfactants) and in biorelevant, special media. The special media reproduces closely the composition of stomach and intestinal tract fluids - bile salts and other relevant physiologically based ingredients; being designed to represent the fed and fasted state in the intestinal cavities – stomach, small intestine, large bowel.

The study plan involved dissolution tests on three categories of samples: (1) raw wild yam DSG standardized extract (SE); (2) raw NLCs containing wild yam SE; (3) finished product capsules containing the NLCs mentioned above and formulation excipients. Assuming that the excipients and the capsule in the formulation of the finished product do not bring a significant intervention on the dissolution of diosgenin, the dissolution tests in special media were drawn up on the finished product – that contains two types of plant extracts: yam extract (DSG) and licorice.

Based on the literature data, available samples, and the chosen dissolution media (conventional or special), the below study plan was established, containing the number of tested samples (Table I, Table II and Table III).

Table I

Tested samples

No.	Code	Tested samples
1	T1 - RWY	raw wild yam extract standardized to 95% DSG (samples)
2	T2 – R Nano-1	Raw NLC-US-DSG-YAM*
3	T3 – R Nano-2	Raw NLC-ULN-DSG-YAM*
4	T4 –R Nano-3	Raw NLC-ULN-DSG-ELD*
5	C1 Nano 1	FP NLC-US-DSG-YAM (S810421)
6	C2 Nano 2	FP NLC-ULN-DSG-YAM (S820421)
7	C3 Nano 3	FP NLC-ULN-DSG-ELD (S060521)

*NLC – nano-lipid capsules, DSG – diosgenin (from standardized wild yam extract), FP – finished product (C-capsule), ULN – Evening Primrose oil, US – Soybean oil, DSG – wild yam extract standardized to 95% DSG, YAM – wild yam extract standardized to 6% DSG, ELD – licorice extract standardized to 10% glycyrrhizic acid

Table II

Study Plan for the dissolution study in conventional dissolution media. The target dissolution parameter was a minimum of 70% dissolved DSG. Dissolution with conventional apparatus HANSON SR8 PLUS +; Test method - Sample reading with HPLC-AGILENT equipment

No.	Conventional dissolution media		Samples to be tested (no.)						
			T1	T2	T3	T4	C1	C2	C3
1	Distilled water		6	1*	1	1	6	6	6
2	Artificial gastric juice (0.1 M HCl)		6	1	1	1	6	6	6
3	Phosphate buffer	pH = 4.5	6	1	1	1	6	6	6
4		pH = 6.8	6	1	1	1	6	6	6
5		pH = 7.2	6	1	1	1	6	6	6
6		pH = 7.5	6	1	1	1	6	6	6
7	Sodium lauryl sulphate	0.5 g/L	6	1	1	1	6	6	6
8		1.0 g/L	6	1	1	1	6	6	6
9		2.0 g/L	6	1	1	1	6	6	6
10	Tween 80	10 g/L	6	1	1	1	6	6	6
11	Cetrimide	4.0 g/L	6	1	1	1	6	6	6

*due to the reduced amount of NLC, a single sample/medium is performed at this stage

Table III

Study plan for the dissolution study in special dissolution media. We tested the formula proposed for further development. Test method - Dissolution with conventional apparatus HANSON SR8 PLUS. Sample reading with HPLC (AGILENT)

No.	Biorelevant dissolution media			Samples to be tested (no.) C3 formula
1	Gastric Fluid	Fasted State Simulated /FaSSGF		6
2		Fed/FEDGAS*	early	pH = 3	6
3			mid	pH = 4.5	6
4			late	pH = 6	6
5	Intestinal Fluid	Fasted State Simulated Intestinal Fluid/FaSSIF		6
6		Fed State Simulated Intestinal Fluid/FeSSIF		6
7	Colonic fluid	Human fasted state colonic fluid/FaSSCoF		6
8		Human fed state colonic fluid/FeSSCoF		6

* stages of stomach emptying

Simulation of gastrointestinal conditions is essential to adequately predict the *in vivo* behaviour of drug formulations, a reasonably accurate estimation of the physiological solubility [37]. To reduce the size and number of human studies required to identify a drug product with appropriate performance in both the fed and fasted states, it is advantageous to be able to pre-screen formulations *in vitro*. The choice of appropriate media for such *in vitro* tests is crucial to correctly forecast the food effect in pharmacokinetics studies.

Biorelevant media simulate gut fluids more accurately than any other dissolution media (Figure 2). They contain components (bile salts, phospholipids and salts) that replicate conditions found in the gastrointestinal tract such as the solubilizing properties, pH and osmolality. The mouth (pH = 6.8), the stomach (pH = 2 - 3) and the intestinal tract (pH = 7) are the main digestive phases. Compositions of different biorelevant media (FaSSIF, FeSSIF and FaSSGF, for example) vary depending upon the location and fed/fasted condition of the fluid they are simulating.
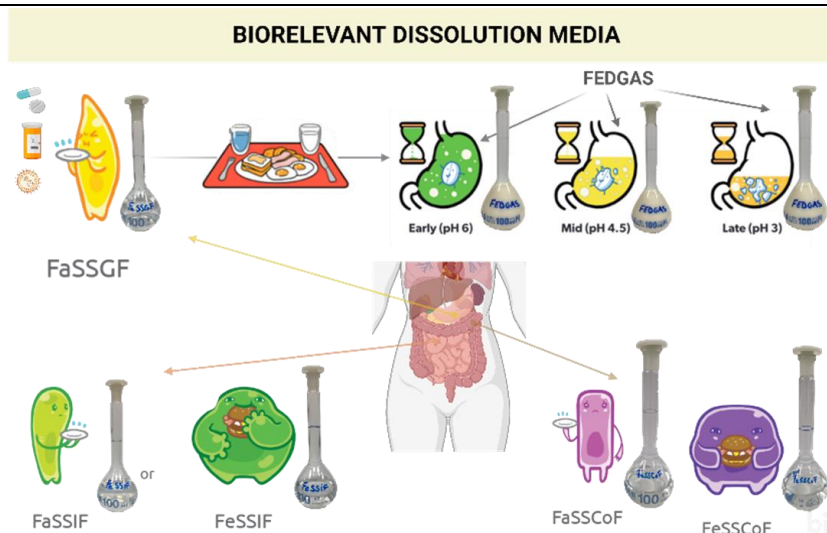
Figure 2.
Graphical representation of the biorelevant dissolution media – FaSSGF – Fasted State Simulated Gastric Fluid; FEDGAS – Fed Gastric Media; FaSSIF – Fasted State Simulated Intestinal Fluid; FeSSIF – Fed State Simulated Intestinal Fluid; FaSSCoF – Human fasted state colonic fluid; FeSSCoF – Human fed state colonic fluid

Materials

Diosgenin. Diosgenin (DSG) (95% purity, extracted from the rhizomes of *Dioscorea villosa*) was obtained from Organic Herb Inc. (Changsha, China). Raw wild yam DSG standardized extract (SE) - *Dioscorea villosa* contains 95% DSG – (3β,25R)-(spirost-5-en-3-ol, $C_{27}H_{42}O_3$) (Figure 3).

Raw NLC-DSG nanoparticles. The raw NLC-DSG nanoparticles were prepared by the melt-emulsification method and high-pressure homogenization, described in previously published research (Biotechnological Laboratoires, Polytechnic University of Bucharest, Romania 2020) [11].

The three NLCs specimens taken in the study were (Figure 3): (1) NLC_US_DSG_YAM – active principle DSG; (2) NLC_ULN_DSG_YAM – active principle DSG; (3) NLC_ULN_DSG_ELD – active principle DSG and licorice; (ULN – Evening Primrose oil, US – Soybean oil, DSG – wild yam extract standardized to 95% DSG, YAM – wild yam extract standardized to 6% DSG, ELD – licorice extract standardized to 10% glycyrrhizic acid).
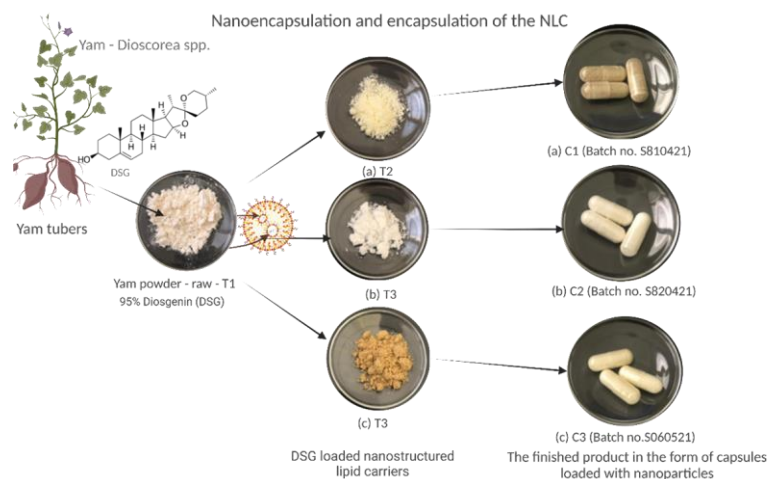

Figure 3.
The finished product in the form of capsules containing:
(a) C1 (Batch no. S810421), (b) C2 (Batch no. S820421), (c) C2 (Batch no. S060521)

The composition of the lipid nanostructured carriers encompasses: (a) the active substance to be nano-encapsulated – the extract of wild yam root (*Dioscorea villosa*) standardized to 95% DSG; (b) the lipid matrix (solid lipids - glyceryl monostearate, cetyl palmitate + vegetable oils - soybean oil/evening primrose oil) and (c) the mixture of surfactants + co-surfactants – reduces surface tension, ensures system stability, being located between the lipid phase and the aqueous phase [29].

The lipid phase consisting of a blend of solid and liquid lipids and the aqueous phase were homogenized under continuous stirring at a sufficiently high temperature so that the lipid part does not solidify. The mixture (pre-emulsion) was submitted to the HSH process (High Shear Homogenizer) followed by a HPH process (High Pressure Homogenizer). The obtained nano-dispersions were cooled at r.t. (room temperature), stored overnight in a freezer and freeze-dried by lyophilization.

The NLCs encapsulating active plant principles obtained as described above were analysed regarding the size of the particles by dynamic light scattering (DLS) method, using a ZetaSizer Nano equipment. Colloidal particles ranging in size between 10 and 1000 nm are known as nanoparticles [39]. The results prove that the NLCs obtained in this study meet the dimension requirements of nanoparticles (Table IV, Figure 4).

Table IV

Mean particle size results for NLCs

Sample	Z-Average (nm)	Standard Deviation	Relative standard deviation
T2 NLC_US_DSG_YAM	107.1	0.5442	0.5083
T3 NLC_ULN_DSG_YAM	119.2	0.6489	0.5442
T4 NLC_ULN_DSG_ELD	162.7	0.8259	0.5076

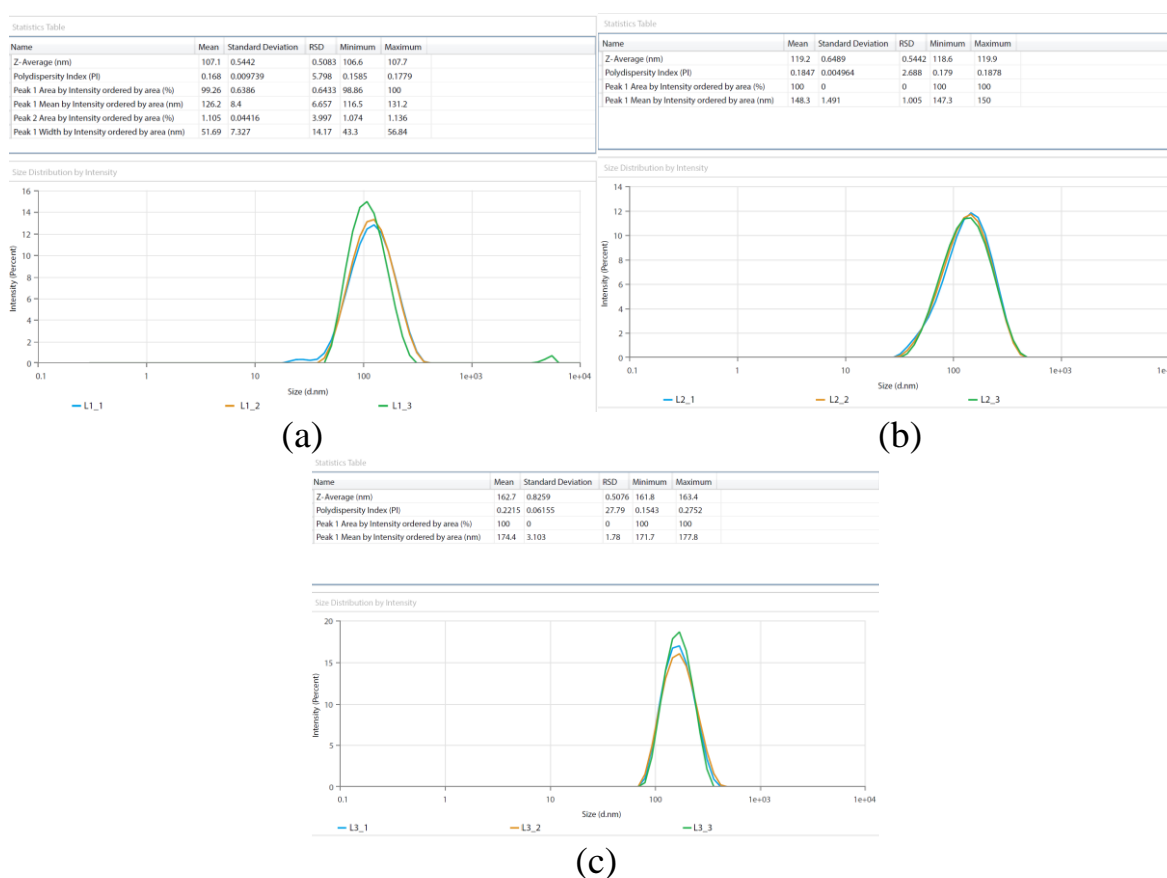(a)   (b)

(c)

Figure 4.
Graphical representation of the size distribution by intensity for:
(a) T2 NLC-US-DSG-YAM, (b) T3 NLC-ULN-DSG-YAM, (c) T4 NLC-ULN-DSG-ELD
(each assay was performed in triplicate)

Finished product containing NLC
We conducted pre-formulation studies to find an excipient to incorporate the oily lipid matrix of NLCs and transform them into a powdery mixture suitable for technological processing. Relevant results were obtained with the spray-dried granular amorphous silica excipient, which was finally selected as the main excipient in the formulation of capsule pharmaceutical form.

The finished product in the oral administration form (hard gelatine capsules) was formulated to contain (a) raw NLC-DSG nanoparticles that incorporate diosgenin and (b) excipients (AC HELCOR® RD Center, Romania) (Table V).
Dissolution media
The dissolution test was carried out according to the USP Apparatus 2 setup using the HANSON SR8PLUS

dissolution tester (Teledyne Hanson Research Ltd., Chatsworth, CA, USA).

Materials used in the dissolution tests, performed in conventional media: distilled water; artificial gastric juice (pH = 1.2); phosphate buffer pH = 4.5; phosphate buffer pH = 6.8; phosphate buffer pH = 7.2; phosphate buffer pH = 7.5; 0.5 g/L, 1.0 g/L, 2.0 g/L sodium lauryl sulphate; 4.0 g/L cetrimide; 10.0 g/L tween 80.

Table V

Formulation of finished product in the form of hard gelatine capsules

Ingredients	Finished product (capsule) C1 (S810421)	Finished product (capsule) C2 (S820421)	Finished product (capsule) C3 (S060521)
	Quantity, %		
T2	79.2	-	-
T3	-	79.2	-
T4	-	-	79.2
Excipient 1	19.8	19.8	19.8
Excipient 2	1.0	1.0	1.0
Total	100	100	100

Materials used in dissolution tests performed in special (biorelevant) media (Biorelevant.com Ltd, London, UK): Fasted State Simulated Gastric Fluid (FaSSGF); Fed Gastric Media pH = 3 (FEDGAS); Fed Gastric Media pH = 4.5 (FEDGAS); Fed Gastric Media pH = 6 (FEDGAS); Fasted State Simulated Intestinal Fluid (FaSSIF); Fed State Simulated Intestinal Fluid (FeSSIF); Human fasted state colonic fluid (FaSSCoF); Human fed state colonic fluid (FeSSCoF).

Reagents. Double distilled water; methanol HPLC; acetic acid HPLC; ethanol; sodium chloride; 37% hydrochloric acid; orthophosphoric acid 85%, HPLC; monopotassium phosphate (KH_2PO_4); monosodium phosphate (NaH_2PO_4 x $2H_2O$); disodium phosphate (Na_2HPO_4 x $12H_2O$); sodium hydroxide (NaOH); cetrimide (N-cetyl-N,N,N-trimethylammonium bromide); tween 80; sodium lauryl sulphate.

Equipment. Analytical balance PRECISA 310 M (Precisa SRL, Iași, Romania), HANSON SR8PLUS dissolution tester (Teledyne Hanson Research Ltd., CA, USA), HPLC-AGILENT system (Agilent Technol. Ltd., Santa Clara, CA, USA) with Chromatographic column: Mediterranea Sea (Teknokroma Analítica SA, Barcelona, Spain, EU), C18, 150 x 4.6 mm, 3 μm.

Analysed parameters. Content of dissolved DSG: Min. 70% (Q), after 60 minutes.

Testing procedure

Dissolution tests. Experimental conditions: Apparatus 1, baskets, Average dissolution volume: 900 mL, Stirring speed: 100 rpm, Temperature: 37 ± 0.5°C, Dissolution time, minutes: 60; Acceptance criteria: Content of dissolved DSG: Min. 70% (Q), after 60 minutes.

Procedure. The test was performed in each media according to the study plan (Table I and Table II). After 60 minutes, 50 mL of dissolution media were withdrawn and filtered (white band filter paper, discarding the first portion and then through a 0.22 μm filter).

Assay of the active ingredient - Diosgenin. Experimental conditions: HPLC-AGILENT high-performance liquid chromatography with a quaternary pump, diode array detector, chromatographic column thermostat oven, standard flow cell, autosampler; Column: Mediterranean Sea, C18, 150 x 4.6 mm, 3 μm; Mobile phase: aceto-nitrile: 0.05% phosphoric acid (90:10, v/v), homogenized and degassed; the column temperature - 30°C; Flow rate: 1 mL/min; UV detection at 206 nm; Injection volume: 100 μL; Evaluation: external standard method. The inter-day and intra-day precision were below 2%. Solutions used in the assay: Standard solution: 11.1 mg DSG was dissolved in methanol, then diluted with the same solvent to 10 mL. 1 mL of this solution was diluted to 100 mL with the corresponding dissolution media (0.0111 mg/mL DSG RS). The chromatographic column was equilibrated with the mobile phase. 100 μL of standard DSG solution and 100 μL of solution from each sample were repeatedly injected; the chromatograms were recorded.

Statistical analysis

The areas of the main peaks in the two chromatograms are measured and the amount of dissolved DSG/ sample, in% was calculated using the formula:

$$Q\,(\%) = (As \times Cs \times 10)/Ast, \quad (1)$$

Q – the amount of dissolved DSG (%); As – the peak area corresponding to the DSG in the sample solution; Cs – a mass of the DSG standard, mg; Ast – the peak area corresponding to the DSG in the standard solution; 10 – dilution factor.

All results were expressed as mean ± SD. Statistical analysis was performed in the aqueous solubility determination, dissolution in conventional media and dissolution in biorelevant media using Excel (Office Microsoft 2010, Albuquerque, NM, USA).

Results and Discussion

The dissolution studies were carried on three categories of samples: (1) wild yam extract standardized to DSG, (2) NLCs containing wild yam extract standardized to DSG (three samples) and (3) finished product formulated as hard gelatine capsule containing NLCs with wild yam DSG standardized extract (3 samples).

The samples mentioned above were tested in two types of dissolution media: (a) conventional and (b)

special (biorelevant) media for the form chosen to be further developed as a finished product based on pharmacological data [11].

Dissolution in conventional media for raw DSG

The first sample to be tested was wild yam plant extract standardized to 95% DSG. When diluting the standard solution with the following dissolution media: distilled water, artificial gastric juice, phosphate buffer pH = 4.5, phosphate buffer pH = 6.8, phosphate buffer pH = 7.2, phosphate buffer pH = 7.5, the precipitation phenomenon occurred, which required the filtration of the solution. Due to this phase, there is a risk that the substance will remain in the filter cartridge, not suitable for dissolution test.

Following the analysis of the chromatograms, we found that no characteristic peak is obtained neither in the case of the standard nor in the case of the sample, DSG didn't dissolve in the conventional media mentioned above (distilled water, artificial gastric juice, phosphate buffer pH = 4.5/6.8/7.2/7.5).

Further dissolution tests were performed in the following media: 0.5 g/L, 1.0 g/L, 2.0 g/L sodium lauryl sulphate (SLS); tween 80 - 10 g/L, cetrimide 4.0 g/L media. The amount of DSG dissolved in the conventional dissolution media, from the wild yam extract standardized to 95% DSG, is presented in Table VI. The recommended media are those that comply with the acceptance criteria of a minimum of 70% dissolved DSG (Table VI).

Table VI

Amount of DSG dissolved from wild yam extract in the studied conventional media

No.	Dissolution media	% dissolved DSG (average)
1	Distilled water	0.0
2	Artificial gastric juice	0.0 (precipitate)
3	Phosphate buffer pH = 4.5	0.0 (precipitate)
4	Phosphate buffer pH = 6.8	0.0 (precipitate)
5	Phosphate buffer pH = 7.2	0.0 (precipitate)
6	Phosphate buffer pH = 7.5	0.0 (precipitate)
7	Sodium lauryl sulphate 0.5 g/L	50.2
8	Sodium lauryl sulphate 1.0 g/L	87.0
9	Sodium lauryl sulphate 2.0 g/L	83.1
10	Tween 80 10 g/L	38.2
11	Cetrimide 4.0 g/L	78.8

As presented above, the only three media in which a minimum of 70% dissolved DSG was obtained, are sodium lauryl sulphate 1.0 g/L, sodium lauryl sulphate 2.0 g/L and cetrimide 4.0 g/L. The explanation could be that these media contain surfactants, which are known to improve the solubility of an active ingredient. The use of surfactants to enhance the dissolution performance of poorly soluble drug products is probably the basic, primary and oldest method. Surfactants reduce surface tension and improve the dissolution of lipophilic drugs in an aqueous medium. They are also used to stabilize drug suspensions.

Conventional media dissolution results NLC containing wild yam extract standardized to DSG dissolution results

The samples included in the dissolution study with conventional media were the nanostructured lipid carrier (NLC) containing wild yam extract standardized to DSG.

Table VII

Amount of DSG dissolved from the raw DSG standardized wild yam extract and raw NLCs containing wild yam extract in conventional media

No.	Conventional dissolution media		Solubility - Dissolved DSG (%) (AVG ± SD)			
			T1 raw DSG extract	T2 NLC-US-DSG-YAM	T3 NLC-ULN-DSG-YAM	T4 NLC-ULN-DSG-ELD
1	Water		0.0	-	-	-
2	Artificial gastric juice		0.0*	-	-	-
3	Phosphate buffer	pH = 4.5	0.0*	-	-	-
4		pH = 6.8	0.0*	-	-	-
5		pH = 7.2	0.0*	-	-	-
6		pH = 7.5	0.0*	-	-	-
7	SLS (g/L)	0.5	50.2 ± 5.32	-	-	-
8		1	87.0 ± 1.04	83.3 ± 1.93	84.4 ± 2.54	83.7 ± 1.62
9		2	83.1 ± 1.24	81.2 ± 1.33	80.2 ± 1.02	81.3 ± 1.17
10	Tween 80	10	38.2 ± 1.12	-**	-**	-**
11	Cetrimide	4	78.8 ± 0,49	71.4 ± 2.29	74.2 ± 2.08	74.9 ± 1.97

* precipitate, ** Because in the first testing stage, on the yam extract, consistent results were obtained in 1.0 g/L and 2.0 g/L sodium lauryl sulphate and 4.0 g/L cetrimide dissolution media, these were taken into the study in the following stages of testing – on NLCs

Given the fact that wild yam extract dissolved only in three types of conventional media, we chose further on 1.0 g/L and 2.0 g/L sodium lauryl sulphate and 4.0 g/L cetrimide as the media for the dissolution of NLCs and the finished product.

The amounts of dissolved DSG from NLCs containing wild yam extract standardized to DSG in the studied dissolution media, met the criteria of specification of minimum 70% DSG released from the initial sample (Table VII).

Dissolution tests on the three forms (capsules) proposed for the final finished product in conventional media

The finished product samples (C1-3), formulated as hard gelatine capsules containing NLC with wild yam extract standardized to DSG were tested in conventional media, and the selected final finished form (C3) dissolution profile was compared with the dissolution in the special dissolution media. The dissolution was carried on the finished product in the same conventional media which ensured good results with the wild yam extract and NLCs, namely 1.0 g/L and 2.0 g/L sodium lauryl sulphate and 4.0 g/L cetrimide.

Dissolution in 1.0 g/L sodium lauryl sulphate media. The amount of DSG dissolved in the 1.0 g/L sodium lauryl sulphate dissolution media from the finished product containing NLC with wild yam extract standardized to DSG, is presented in Table VIII.

Dissolution in 2.0 g/L sodium lauryl sulphate media. The amount of DSG dissolved in the 2.0 g/L sodium lauryl sulphate dissolution media, from the finished product containing NLC with wild yam extract standardized to DSG, is presented in Table IX.

Dissolution in 4.0 g/L cetrimide media. The amount of DSG dissolved in the Cetrimide 4.0 g/L dissolution media, from the finished product containing NLC with Wild Yam extract standardized to DSG, is presented in Table X.

Table VIII

Amount of DSG dissolved in 1.0 g/L sodium lauryl sulphate media

Sample no.	C1 (S810421)	C2 (S820421)	C3c (S060521)
	% dissolved DSG		
1	81.5	81.5	80.3
2	80.4	80.4	79.9
3	83.6	78.8	78.4
4	79.8	82.6	80.5
5	79.6	80.9	81.4
6	80.4	81.9	80.1
Average	80.8	81.0	80.1
SD	1.49	1.33	0.98
RSD	1.84	1.64	1.23

Table IX

Amount of DSG dissolved in 2.0 g/L sodium lauryl sulphate media

Sample no.	C1 (S810421)	C2 (S820421)	C3 (S060521)
	% dissolved DSG		
1	77.8	76.8	79.7
2	79.1	76.9	78.8
3	80.5	77.4	80.9
4	81.3	79.8	80.4
5	78.4	80.1	79.4
6	79.3	76.9	78.6
Average	79.4	77.9	79.6
SD	1.30	1.54	0.90
RSD	1.64	1.98	1.13

Table X

Amount of DSG dissolved in Cetrimide 4.0 g/L media

Sample no.	C1 (S810421)	C2 (S820421)	C3 (S060521)
	% dissolved DSG		
1	75.6	77.9	73.9
2	74.8	76.8	72.5
3	73.9	75.4	74.1
4	78.4	77.3	73.5
5	76.6	74.9	74.3
6	73.5	75.9	73.9
Average	75.4	76.3	73.7
SD	1.83	1.16	0.64
RSD	2.42	1.52	0.88

The amounts of dissolved DSG from capsules containing NLC with wild yam extract standardized to DSG in the studied dissolution media are centralized in Table XI.

1.0 g/L sodium lauryl sulphate, 2.0 g/L sodium lauryl sulphate and 4.0 g/L cetrimide are the suitable conventional media chosen and introduced in the quality specification as a routine dissolution test for a finished product containing NLC with wild yam extract standardized to 95% DSG encapsulated in nano lipid carrier. The comparative analysis is provided in Figure 5.

Table XI

The amount of DSG dissolved from the finished forms taken into the study

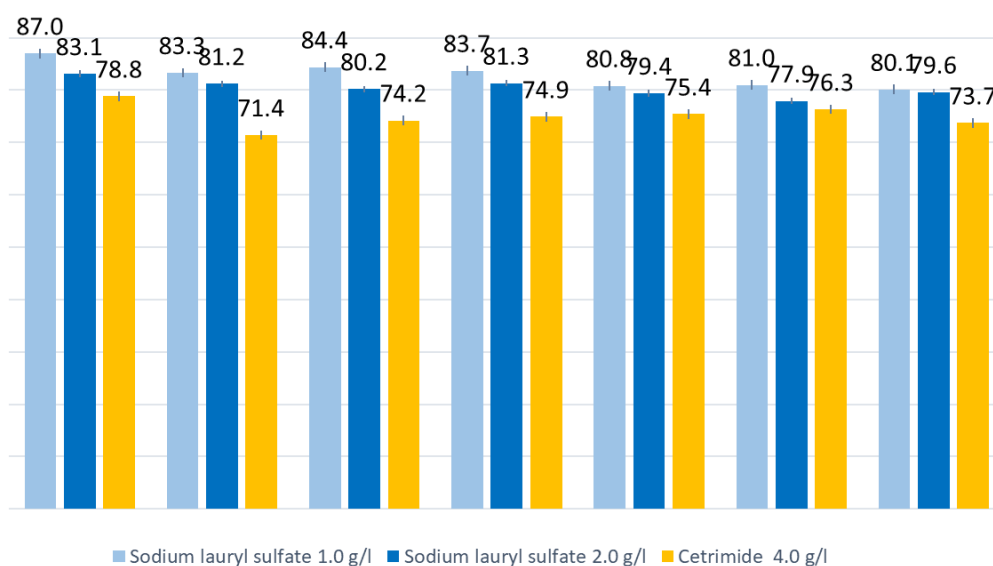| Dissolution media	C1 (S810421)	C2 (S820421)	C3 (S060521)
	% dissolved DSG (average)		
Sodium lauryl sulphate 1.0 g/L	80.8	81.0	80.1
Sodium lauryl sulphate 2.0 g/L	79.4	77.9	79.6
Cetrimide 4.0 g/L	75.4	76.3	73.7

Figure 5.

Final comparative dissolution results in the conventional dissolution media, % DSG for all the tested samples – raw wild yam DSG standardized (n = 1), raw NLC's (n = 3) and finished forms (n = 3)

Finished NLC product dissolution in special biorelevant media

In the following stage of the study, we chose to test the most suitable formula for further development of a final finished product based on dissolution tests and preliminary pharmacological data [11]; dissolution tests in special bio relevant media were carried out on the same samples on which dissolution was tested in conventional media, namely a finished product formulated as hard gelatine capsule containing a nanostructured lipid carrier which incorporates wild yam extract standardized to DSG and excipients.

Dissolution in Fasted State Simulated Gastric Fluid (FaSSGF) media. The amount of DSG dissolved in the FaSSGF dissolution media from the capsules containing NLC with DSG, is presented in Table XII. The results of the dissolution in the media that simulates the gastric fluids before a meal do not meet the acceptance criteria of Min 70% dissolved DSG.

Dissolution in Fed Gastric Media (FEDGAS) media. The amount of DSG dissolved in the FEDGAS dissolution media at three different pH values from the capsules containing NLC with DSG, is presented in Table XIII.

Table XII

Amount of DSG dissolved in FaSSGF media from the tested formula (C3)

Sample no.	FaSSGF media
	% dissolved DSG
1	0.0
2	0.0
3	0.0
4	0.0
5	0.0
6	0.0
Average	0.0
SD	0.0
RSD	0.0

The results of the dissolution in the media that simulate the stomach fluids after a meal meet the acceptance criteria of Min 70% dissolved DSG only for the FEDGAS media at a pH value of 6, FEDGAS media at pH values of 3 and 4.5 do not meet the acceptance criteria.

Table XIII

Amount of DSG dissolved in FEDGAS media from the tested formula (C3)

Sample no.	FEDGAS		
	pH = 3	pH = 4.5	pH = 6
	% dissolved DSG		
1	0.0	55.1	79.7
2	0.0	60.1	75.9
3	0.0	60.9	75.3
4	0.0	59.4	78.7
5	0.0	56.9	77.4
6	0.0	63.4	76.8
Average	0.0	59.3	77.3
SD	0.0	2.95	1.67
RSD	0.0	4.97	2.16

Dissolution in Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) media. The amount of DSG dissolved in the FaSSIF and FeSSIF dissolution media from the capsules containing NLC with DSG, is presented in Table XIV.

Table XIV

Amount of DSG dissolved in FaSSIF and FeSSIF media from the tested formula (C3)

Sample no.	FaSSIF media	FeSSIF media
	% dissolved DSG	
1	75.5	85.6
2	75.4	84.9
3	71.2	81.8
4	70.9	85.2
5	70.0	86.2
6	70.2	81.9
Average	72.2	84.0
SD	2.56	2.02
RSD	3.54	2.41

The results of the dissolution in the media that simulate the intestinal fluids before and after a meal meet the acceptance criteria of Min 70% dissolved DSG.

Dissolution in Human fasted state colonic fluid (FaSSCoF) and Human fed state colonic fluid (FeSSCoF) media. The amount of DSG dissolved in the FaSSCoF and FeSSCoF dissolution media, from the capsules containing NLC with DSG, is presented in Table XV.

Table XV

Amount of DSG dissolved in FaSSCoF and FeSSCoF media from the tested formula (C3)

Sample no.	FaSSCoF media	FeSSCoF media
	% dissolved DSG	
1	59.7	31.1
2	59.2	35.2
3	60.4	27.4
4	61.0	30.3
5	57.2	35.8
6	57.1	34.6
Average	59,1	32,4
SD	1.63	3.33
RSD	2.76	10.27

Fasted/Fed State Simulated Intestinal Fluid (FaSSIF/FeSSIF) and Fed Gastric Media pH 6 (FEDGAS) are the suitable special media chosen for the dissolution test for the research studies and development of a solid dosage form (hard gelatine capsule) containing an NLC with DSG as the active ingredient (Figure 6). The results of the dissolution in the media that simulate the colonic fluids before and after a meal do not meet the acceptance criteria of a minimum of 70% dissolved DSG after 60 minutes.
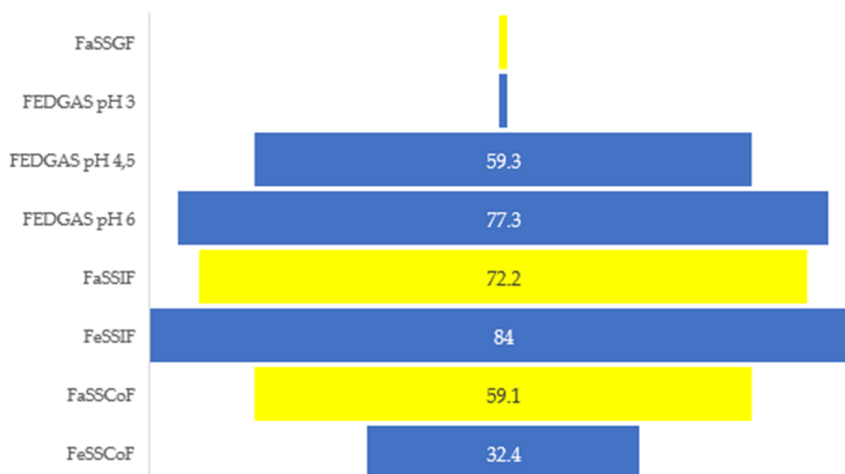

Figure 6.

Dissolution of finished formula C3 in the simulated gut fluids, % DSG (yellow-fasted, blue-fed)

Dissolution studies were carried out on three categories of samples: wild yam extract standardized to DSG, nanostructured lipid carrier (NLC) with encapsulated wild yam extract standardized to DSG, and a finished product formulated as a hard gelatine capsule that contains NLC with Wild Yam extract standardized to DSG.

Two different types of dissolution media were used: special dissolution media, modelling the *in vivo* dissolution behaviour of the final oral solid dosage form containing NLCs – to see the optimal place and time (fed or fasted) for the absorption of diosgenin encapsulated in the nano lipidic structure and in conventional dissolution media in order to determine which media to select for the final quality specification of the finished product for routine dissolution tests (QC test).

Analysing the dissolution data from special media that simulate human gastrointestinal fluids, conventional media with similar pH were chosen for routine laboratory analysis of the finished product. It can be seen in the Table XVI that dissolution results in cetrimide (conventional media – pH 5.3) were similar with results in Fed State Simulated Intestinal Fluid (special media – pH 5), and results in 1.0 - 2.0 g/L sodium lauryl sulphate (conventional media – pH 6.7 - 7.0) were similar with results in Fed Gastric Media and Fasted State Simulated Intestinal Fluid (special media pH 6 - 6.5). The comparative dissolution results are presented in Table XVI.

Table XVI

Comparative dissolution results: conventional *versus* special media (final formula C3)

No.	Dissolution media	pH	Finished product (capsule) C3 formula (S060521) Average dissolved diosgenin (%)
1	Fasted State Simulated Gastric Fluid /FaSSGF (SM)	1.6	0
2	Fed Gastric Media pH 3 /FEDGAS (SM)	3	0
3	Fed Gastric Media pH 4,5 /FEDGAS (SM)	4.5	59.3
4	Fed State Simulated Intestinal Fluid /FeSSIF (SM)	5	84.0
5	Cetrimide 4.0 g/L (CM)	5.3	73.7
6	Human fed state colonic fluid /FeSSCoF (SM)	6	32.4
7	Fed Gastric Media pH 6 /FEDGAS (SM)	6	77.3
8	Fasted State Simulated Intestinal Fluid /FaSSIF (SM)	6.5	72.2
9	Sodium lauryl sulphate 1.0 g/L (CM)	6.7	80.1
10	Sodium lauryl sulphate 2.0 g/L(CM)	7.0	79.6
11	Human fasted state colonic fluid /FaSSCoF (SM)	7.8	59.1

* CM – Conventional media, SM – Special media

From the conventional media used, DSG dissolved only in three: 1.0 g/L sodium lauryl sulphate, 2.0 g/L sodium lauryl sulphate and 4.0 g/L cetrimide. DSG is known to be a poorly soluble substance. DSG is dissolved in these media as they contain surfactants that improve an active ingredient's solubility.

In conventional media, less DSG was dissolved from the finished product (at this stage, DSG was encapsulated in the nano lipid structure) compared with the wild yam extract alone. This may be because excipients of the finished product have a minor interference with nano DSG (they form a shell around DSG, trapping it) and do not allow it to pass through the mesh of the basket in the dissolution media. 1.0 g/L Sodium lauryl sulphate, 2.0 g/L sodium lauryl sulphate and 4.0 g/L cetrimide are suitable to be chosen as conventional dissolution media for future dissolution tests according to the quality specification of the finished product. Few studies investigated the dissolution profiles of diosgenin formulated as a nano product [38] in the form of the nanocrystal, confirming a better biodisponibility of the nanoproduct compared to the bulk DSG form [38], and no study investigated the dissolution characteristics of the NLC containing DSG.

The other part of the study focused on dissolution in biorelevant media (special media), which can enable drug developers to identify test formulations with superior dissolution profiles and help establish a relationship between the biopharmaceutical properties of an oral drug and *in vivo* absorption. Looking at the dissolution result of the finished product in special media, we can draw few remarks. The obtained results met the acceptance criteria of min. 70% dissolved DSG in only three media out of eight - Fasted State Simulated Intestinal Fluid (FaSSIF), Fed State Simulated Intestinal Fluid (FeSSIF) and Fed Gastric Media pH 6 (FEDGAS). DSG did not dissolve in acidic media: Fasted State Simulated Gastric Fluid – pH 1.6 and Fed Gastric Media in the late stage of stomach emptying – pH 3.The best dissolution results were obtained in media with pH values between 5 - 6.5: Fed Gastric Media in the early stage of stomach emptying and Fasted and Fed State Simulated Intestinal Fluid, with a present, but reduced dissolution in the fasting Colonic Simulated Fluid. The nano-encapsulated DSG travels through the stomach and dissolves in the intestinal tract, where its' absorption occurs.

The present study has several limitations that the authors acknowledged. The finished forms tested were restricted to the conventional media that showed a dissolution profile of over 70% in the raw wild yam dissolution tests. Another limitation was the small sample size in the raw NLCs dissolution tests due to raw material limitations (lab batch). Another limitation of the perspective is the testing of a single final form – capsules – comprising of DSG and licorice

extract, formulated in evening primrose oil; this gives the views for further research on the (1) bio-relevant media dissolution testing on the following NLCs samples and (2) *in vivo* pharmacokinetic data reflecting the *in vitro* data from the present study.

Conclusions

The intestinal environment after a meal is the optimal absorption place and time of the active principle diosgenin encapsulated in the nano lipid structure, measured in biorelevant dissolution media simulating the physiological *in vivo* conditions.

The recommended dissolution media for the routine laboratory analyses included in the specification of the finished product for scale-up interphasic tests, with a pH close to that of the special media, are 1.0 g/L sodium lauryl sulphate, 2.0 g/L sodium lauryl sulphate and 4.0 g/L cetrimide, the results obtained in this media complying with the imposed minimal limit of 70% dissolved DSG.

The recommended pharmaceutical form for products containing diosgenin encapsulated in a nano lipidic structure is delayed-release (gastro-resistant), and the optimal absorption of the active principle takes place at the upper intestinal level.

Acknowledgement

Conflict of interest
The authors declare no conflict of interest.

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