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Biocompatible lipid nanocarriers for improved skin delivery of fluocinolone acetonide: physicochemical and *in vitro* performances

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Abstract

In order to improve the delivery of topical corticosteroids into inflammatory skin lesions while reducing the likelihood of adverse effects, lipid nanocarriers have received increasing attention. Hence, this study aimed to develop biocompatible nanoemulsions (NEs) and nanostructured lipid carriers (NLCs) as carriers for fluocinolone acetonide (FA) by carefully optimizing the formulation and process parameters. After an analysis of the relevant physicochemical parameters and stability testing, *in vitro* release and permeation tests were performed to evaluate whether the nanocarriers affected the penetration of FA into/through the skin compared to a conventional reference product (Sinoderm® cream). The developed NEs exhibited satisfactory physicochemical properties (droplet size <200 nm, PDI<0.2, ZP>|-30| mV, pH ~ 4.75) and long-term stability. Although the developed NLCs initially had satisfactory properties, gelation was observed within 3 months of storage, implying that further formulation testing is required to resolve the limited stability of these systems. *In vitro* release/permeation findings suggest that the developed nanocarriers (especially NEs) provide better delivery of FA into/though the skin compared to the Sinoderm® cream. Therefore, a lecithin-based NE with a 10% lipid phase (medium-chain triglycerides/oleic acid 3:1) is a promising strategy for improved delivery of FA to the inflamed skin, allowing for ease of application, especially to larger skin surfaces and hairy regions.

Key words: fluocinolone acetonide, nanoemulsions, nanostructured lipid carriers, dermal delivery, particle size

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Introduction

Fluocinolone acetonide (FA) is a synthetic glucocorticoid commonly utilized to treat different inflammatory skin conditions such as *alopecia areata*, psoriasis and various forms of dermatitis, keloids, or hypertrophic scarring (1). According to the European classification system for corticosteroids, FA belongs to group III, which includes highly potent corticosteroids (2, 3). Although there are several conventional topical products with FA on the market, physical difficulties in applying the treatment, lack of visible effect, and, most importantly, numerous side effects lead to poor patient adherence and limit their efficacy. As a result, intensive research has concentrated on the design of nanotechnology-based drug delivery systems to ensure targeted delivery to the skin and enhance the benefit-risk ratio of topical corticosteroids such as FA (4-6).

Among the various proposed nanocarriers, nanoemulsions (NEs) and nanostructured lipid carriers (NLCs) may offer many benefits, including high solubilization capacity for lipophilic drugs (e.g., FA), satisfactory kinetic stability, low skin irritation potential, and relative ease of preparation and scaling (7, 8). Likewise, improved delivery of the incorporated drug into/through the skin can be attributed to an enhanced concentration gradient of the drug, penetration of the nanodroplets/nanoparticles into the hair follicles and their accumulation. Besides, surfactants used for stabilization may disrupt the skin barrier and consequently lead to improved penetration of the drug into/through the skin (7, 8).

In addition, depending on the formulation composition, NEs and NLCs may provide sustained drug release (maintaining therapeutic drug levels over a longer period of time) and reduce the adverse effects of the incorporated drug (9).

The proper selection of excipients, especially the type and proportion of lipids (i.e., liquid lipid(s) for NEs or a mixture of solid and liquid lipids for NLCs) and stabilizers/surfactants, is critical to the quality and stability of these nanocarriers and to the effective penetration of incorporated drugs into/through the skin. In other words, to develop stable lipid nanocarriers such as NEs and NLCs that allow satisfactory dermal delivery of FA, a balance must be struck between the solubility of FA in a lipid phase, the long-term stability of the formulations overall, and their multifaceted interactions with the skin. In addition, the safety profile of surfactants/stabilizers must be considered, as they can cause irritation and other adverse effects on the skin. Oleic acid is a very interesting excipient because it can act as a liquid lipid, drug solubilizer, penetration enhancer, and system stabilizer (which can prevent and/or delay Ostwald ripening (10, 11)). However, there are a limited number of studies addressing the use of oleic acid in the formulation of NEs and NLCs loaded with corticosteroids such as FA.

Therefore, the aim of this research was to develop biocompatible lipid nanocarriers (NEs and NLCs) for improved dermal delivery of FA by varying different formulation (type and content of lipids) and process parameters (number of homogenization cycles). The promising lipid nanocarriers were characterized in terms of droplet/particle size and size distribution, pH, zeta potential and electrical conductivity, and their stability was

evaluated during 12 months of storage at room temperature. After a detailed physicochemical analysis, the impact of the diverse inner structure of the developed vehicles (NEs vs. NLCs) on the *in vitro* release of FA through the synthetic membranes and the *in vitro* permeation of FA through the porcine ear epidermis was investigated. The developed lipid nanocarriers (NEs and NLCs) were compared with the marketed cream formulation (Sinoderm®, Galenika AD, Serbia) as a reference to gain insight into the potential therapeutic benefits of these innovative nanoformulations.

Materials and methods

Materials

Placebo and FA-loaded nanoformulations were prepared using the following excipients: medium chain triglycerides (MCT) (Saboderm TCC,-Sabo S.p.A, Italy), oleic acid (Lipoid GmbH, Germany), hydrogenated palm oil (Softisan® 154) (IOI Oleo GmbH, Germany), soybean oil (Lipoid GmbH, Germany), castor oil (Sigma Aldrich Laborchemikalien GmbH, Germany), propylene glycol monocaprylate (Capryol® 90, Gattefossé, France), egg lecithin (Lipoid® E80, Lipoid GmbH, Germany), polysorbate 80 and butylhydroxytoluene (BHT) (Sigma Aldrich Laborchemikalien GmbH, Germany), sodium chloride (Sigma Aldrich Laborchemikalien GmbH, Germany), potassium dihydrogen phosphate (Sigma Aldrich Laborchemikalien GmbH, Germany), disodium hydrogen phosphate dodecahydrate (Sigma Aldrich Laborchemikalien GmbH, Germany) and ultrapure water (TKA GenPure, TKA Wasseranfbereitungssysteme GmbH, Germany). Fluocionolone acetonide (FA) was kindly donated by Galenika AD (Belgrade, Serbia).

Methods

Solubility studies

The solubility of FA was investigated in different oils (Capriol® 90, soybean oil, castor oil, MCT) and oil mixtures (oleic acid:MCT at ratios 1:1 and 1:3 w/w). In Erlenmeyer flasks, the excess amount of FA was added to each oil and mixed. To achieve equilibrium, the resulting mixtures were maintained at 25 ± 2 ˚C on an orbital shaker for 24 hours. (IKA® KS 260 basic, IKA® Werke GmbH & Co. KG, Staufen, Germany). Subsequently, the mixtures were centrifuged (Centrifuge MPW-56; MPW Med. Instruments, Warszawa, Poland) at 3000 rpm for 30 min. The concentration of FA in the obtained supernatants was determined using high-performance liquid chromatography-mass spectrometry (HPLC-MS) (see below).

Preparation of tested lipid nanocarriers

The hot high-pressure homogenization method (EmulsiFlex C3, Avestin Inc, Ottawa, Canada) was used for the preparation of the tested lipid nanocarriers. Concisely, the water phase and the lipid phase were prepared separately, before homogenization. The lipid phase containing various lipids, egg lecithin, and BHT was heated to 50–85°C,

depending on the lipid used. The water phase containing ultrapure water and polysorbate 80 was heated to the temperature of the oil phase with stirring. Then, the mixture obtained by adding the oil phase to water phase was further pre-homogenized with a rotor-stator homogenizer (IKA Ultra-Turrax® T25 digital, IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 8000 rpm for 5 minutes. Following that, the coarse emulsions were then homogenized for 5-10 homogenization cycles at 800 bars and 50°C. For drug-loaded formulations, FA was added by dissolving in the mixture of lipids and egg lecithin, before emulsification with the water phase. All the prepared formulations were filled into glass vials, tightly closed with crimp cups and stored at room temperature (25 ± 2 ºC). Physicochemical characterization was carried out 24 hours after the preparation and during 12 months of storage at room temperature. All measurements were performed in triplicate. The detailed composition of the lipid nanocarriers studied is shown in Table I.

Table I Composition of the investigated lipid nanocarriers

Tabela I Sastav ispitivanih lipidnih nanonosača

Composition (%, w/w)	**NE10%/ NE10%-FA**	**NE20%/ NE20%-FA**	**NLC10%/ NLC10%-FA**	**NLC20%/ NLC20%-FA**
Oil phase				
MCT	7.5	15	-	-
Hydrogenated palm oil (Softisan® 154)	-	-	7.5	15
Oleic acid	2.5	5	2.5	5
Butylhydroxytoluene	0.05	0.05	0.05	0.05
Egg lecithin	1.5	2	1.5	2
Fluocinolone acetonide	-/0.025	-/0.025	-/0.025	-/0.025
Aqueous phase				
Polysorbate 80	1.5	2	1.5	2
Ultrapure water to	100	100	100	100

*MCT-medium chain triglycerides

Particle size analysis

The mean droplet/particle size (intensity weighted mean diameter (Z-ave)) and corresponding polydispersity index (PDI) of the tested lipid nanocarriers were measured using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK). Before measurements, the tested samples were diluted with ultrapure water (1:500, v/v). Measurements were carried out at 25 ± 2 ºC and a fixed scattering angle of 90º using a He-Ne laser at 633 nm.

Zeta potential analysis

The zeta potential (ZP) was determined using the Zetasizer Nano ZS90, by measuring the electrophoretic mobility of nanodroplets/nanoparticles in the electric field which is then converted to the zeta potential (ZP) via appropriate software. Prior to measurements, samples were diluted with ultrapure water (1:500, v/v).

pH and electrical conductivity measurements

The pH value of tested lipid nanocarriers was measured at 25 ± 2 °C using a HI9321 pH meter (Hanna Instruments Inc., Portugal). Electrical conductivity was measured at the same temperature by a CDM230 Conductivity Meter (Radiometer, Denmark).

Stability study

The physicochemical stability of the developed nanocarriers stored at room temperature (25 ± 2 °C) was monitored for one year, analyzing the relevant parameters e.g., droplet/particle size, polydispersity index, pH, zeta potential and electrical conductivity. In addition, all formulations were visually examined for the changes in the structure and/or phase separation.

In vitro release and permeation studies

In vitro release and permeation tests were performed using modified Franz diffusion cells (Gauer Glas, Germany) with a receptor volume of 12 ml. A mixture of phosphate-buffered saline (pH 7.4) and ethanol 96% v/v (80:20, v/v) was used as the receptor fluid (the exact composition of phosphate-buffered saline (pH 7.4): 8g Sodium chloride, 0.19g Potassium dihydrogen phosphate, 2.38g Disodium hydrogen phosphate dodecahydrate).

Polycarbonate membranes (0.1 μm) (Nuclepore™, Whatman, Maidstone, UK) and heat-separated porcine ear epidermis were used to test *in vitro* release and permeation of FA, respectively. The porcine ear epidermis was prepared using the protocol previously described by Ilić and co-authors (12). The experiments were performed under infinite dose conditions – 1000 μl of the selected NEs and NLCs, or 1 g of the reference Sinoderm® cream (Galenika AD Serbia), were applied to the membranes in the donor chambers, which were then capped with the Parafilm™. The temperature was maintained at 32.0 ± 0.5°C and the stirring speed at 500 rpm. At appropriate time intervals, aliquots of 600 μL were taken and swapped for the same volume of fresh, prewarmed medium. The concentration of FA in the samples was determined by the HPLC-MS method.

HPLC-MS method

The determination of FA in the supernatants/aliquots obtained during the solubility study and the *in vitro* release and permeation testing was performed using a HPLC-MS method. Analyses were conducted by the ACELLA UHPLC chromatograph (Thermo Fisher Scientific Inc., Madison, WI, USA) coupled to the triple quadrupole mass spectrometer TSQ Quantum Access MAX (Thermo Fisher Scientific Inc., Madison, WI, USA) with heated electrospray ionization (HESI) interface. Chromatographic separation

was achieved using Zorbax Extend C18 (150 mm x 4.6 mm, 5µm; Agilent technologies) at 25°C. For isocratic elution, a mixture of methanol and 0.1% formic acid (70:30, v/v) was used at a flow rate of 500 µl/min. For the quantification of FA, an [M+H]+ ion was selected (453.1).

Statistical analysis

The results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using the Student's t-test or one-way analysis of variance (ANOVA) followed by the Tukey post hoc test, depending on the number of data groups being compared (PASW Statistics version 23.0, SPSS Inc., Chicago, USA). The normality of the data was tested using the Shapiro–Wilk test. Statistical significance was set at $p < 0.05$.

Results and discussion

Selection of excipients and processing conditions

FA is a hydrocortisone derivative exhibiting a molecular weight of 452.5 g/mol, poor aqueous solubility (0.05 mg/ml), a log P value of 2.48, and a predicted pKa of 13.9, which is classified in the BCS class II with low solubility and high permeability (13). Considering the low water solubility of FA, it is important to dissolve it at a therapeutic concentration (0.025%) in the lipid core of NEs and NLCs to successfully utilize its advantages in drug delivery through the skin. Therefore, the solubility of FA in various oils and oil mixtures was firstly tested. The highest solubility of FA was obtained in propylene glycol monocaprylate (Capriol® 90) as a liquid lipid (Table II), which can act both as a liquid lipid and as a nonionic water-insoluble co-surfactant (9). Despite numerous variations in the formulation composition (proportion and type of the solid lipid and liquid lipids, proportion of lecithin and/or polysorbate 80), no NEs and NLCs could be prepared with Capriol® 90 as a liquid lipid (alone or in combination with other lipids) (data not shown). Therefore, the next oil blend (MCT-oleic acid blend in a 3:1 ratio) was selected for the development of NEs, which has a high solubilization capacity for FA (Table II). At the same time, Softisan® 154-oleic acid blend in the ratio of 3:1 was used to develop NLCs. It has been shown that oleic acid can perturbate the *stratum corneum* lipid bilayers and increase their fluidity, due to the cis-double bond at C9 which causes a kink in the alkyl chain, allowing improved drug delivery into the *stratum corneum* as well as hair follicles (10, 11). Considering that the content of the lipid phase can influence the solubilization capacity and overall performance of NE and NLC for the skin (higher content of the lipid phase, higher skin occlusion factor, and improved drug delivery to the skin (14)), different contents of the lipid phase (10% and 20%) were tested.

Another crucial aspect that has to be carefully considered in the development of lipid nanocarriers is the selection of appropriate stabilizers/surfactants. Due to their excellent biocompatibility, ability to enhance solubilization of the drug and its penetration into/through the skin, natural lecithins are generally the first choice among the numerous

emulsifiers used to stabilize nanoformulations (15, 16). Since it was not possible to prepare physically stable FA-loaded NEs and NLCs stabilized by egg lecithin alone, the hydrophilic surfactant polysorbate 80 was added to achieve a more balanced HLB value within the interfacial surfactant mixture, so as to promote interfacial film curvature and nanocarrier stability (15). Table I shows the composition of the lipid nanocarriers studied. To evaluate the impact of FA on the physicochemical properties of the tested nanocarriers, corresponding placebo samples were prepared and characterized simultaneously.

Table II Solubility of fluocinolone acetonide in different oils and oil mixtures

Tabela II Rastvorljivost fluocinolonacetonida u različitim uljima i smešama ulja

Excipients	Solubility (mg/ml)
Propylene glycol monocaprylate (Capriol® 90)	3.6756
Medium chain triglycerides	0.6556
Soybean oil	0.1031
Castor oil	0.0330
Oleic acid	0.6762
Oleic acid:medium chain triglycerides 1:1	0.4784
Oleic acid:medium chain triglycerides 1:3	0.6813

Among the various methods for preparing lipid nanocarriers, hot-high pressure homogenization is the method of choice because it is relatively simple and the droplet/particle size distribution can be easily regulated (17). However, it should be noted that in addition to the formulation variables, the process parameters (temperature, homogenization pressure, number of cycles) can also significantly influence the droplet/particle size and stability of the tested lipid nanocarriers. The homogenization pressure was set at 800 bar to avoid metal contamination that can occur at higher pressures due to the higher wear of the homogenizer valves (16, 18). On the other hand, the effect of the homogenization cycle number on droplet/particle size and PDI of both NEs and NLCs containing 10% and 20% lipid phase with and without FA was experimentally investigated (Figure 1). The differences in droplet/particle size and polydispersity index were small and statistically insignificant between 5 and 10 homogenization cycles for the mentioned formulations. However, 10 homogenization cycles were selected to ensure the same preparation conditions for all tested samples (better reproducibility was also considered).

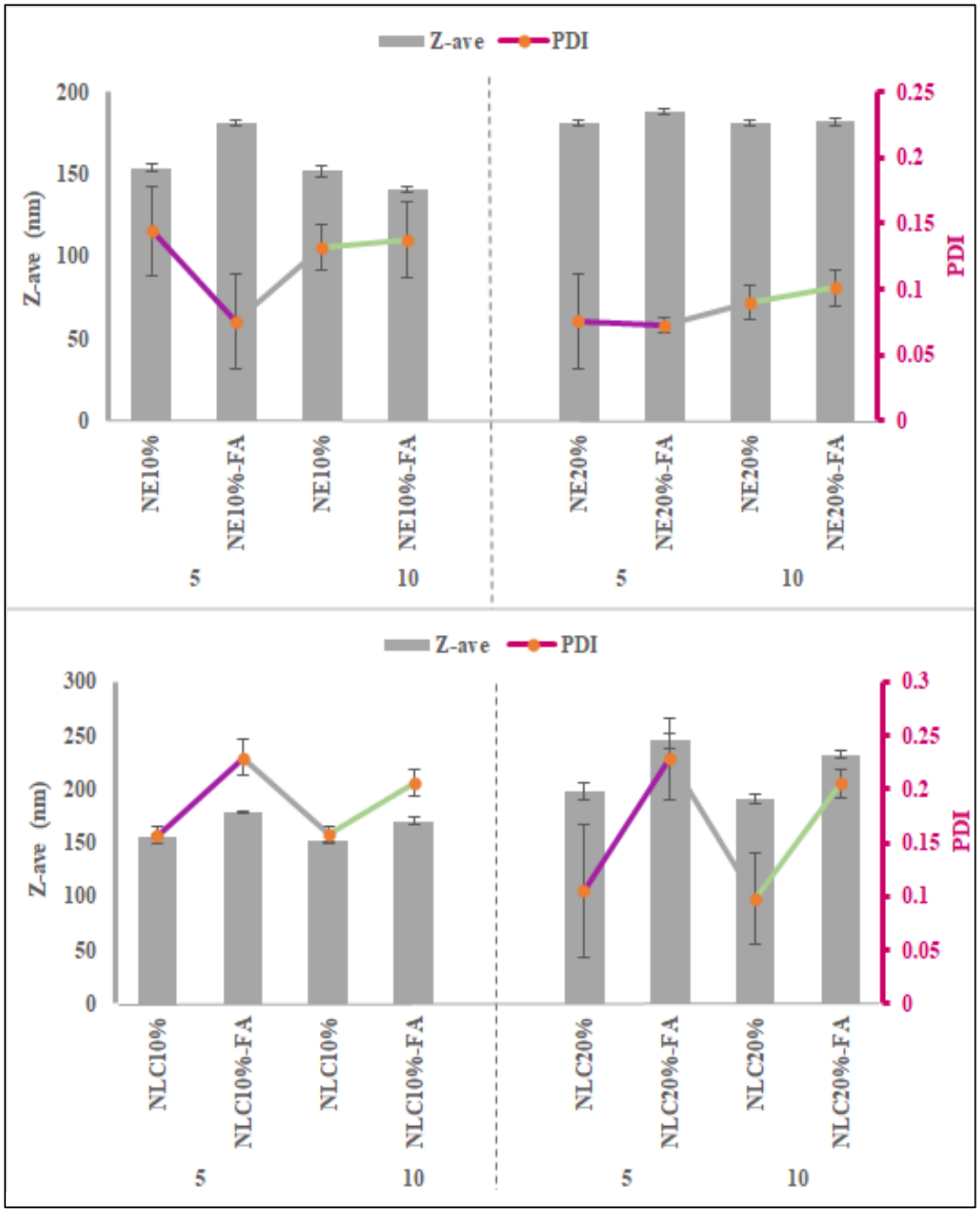

Figure 1. The influence of the number of homogenization cycles (5/10) on the droplet/particle size (Z-ave) and polydispersity index (PDI) of the developed lipid nanocarriers with 10% and 20% lipid phase

Slika 1. Uticaj broja (5/10) ciklusa homogenizacije na veličinu kapi/čestica (Z-ave) i polidisperzni indeks (PDI) razvijenih lipidih nanonosača sa 10% i 20% masne faze

Physicochemical characterization of developed lipid nanocarriers

With the exception of the NLC20% lipid phase formulation loaded with FA, which began to gel 7 days after preparation, all other samples were very fluid with a milky white appearance and bluish coloration. With the exception of the NLC20%-FA formulation, the droplet/particle size was under 200 nm, with low PDI (<0.2). As expected, a significant upsurge in droplet/particle size was detected for both lipid nanocarriers tested when the lipid phase content was increased (Table III). Considering that the same process parameters were used, less dispersion energy per lipid unit is available when the total amount of lipids is increased, resulting in an increase in particle size (9, 19). Interestingly, when comparing the particle size of placebo NE and NLC with 10% and 20% lipid phase, no remarkable difference was observed, suggesting that the incorporation of solid lipid (hydrogenated palm oil, Softisan® 154) had no effect on particle size. However, the same trend was not observed for FA-loaded formulations – the particle size of NLCs with 10% and 20% lipid phase was higher than that of NEs with the same lipid content – probably due to the inherent instability of these samples (see below).

Table III Changes in droplet/particle size (Z-ave) and polydispersity index (PDI) of tested lipid nanocarriers during one year of storage at room temperature (25±2°C)

Tabela III Promene veličine kapi/čestica (Z-ave) i polidisperznog indeksa (PDI) ispitivanih lipidnih nanonosača tokom godinu dana čuvanja na sobnoj temperaturi (25±2°C)

Formulation	Z-ave	PDI
Initially		
NE10%	152.0±3.4	0.132±0.017
NE10%-FA	140.1±1.6	0.138±0.029
NE20%	180.6±1.8	0.090±0.013
NE20%-FA	181.6±2.3	0.101±0.014
NLC10%*	151.3±0.2	0.158±0.008
NLC10%-FA*	170.0±3.6	0.206±0.012
NLC20%*	190.6±4.6	0.098±0.043
NLC20%-FA*	231.8±3.6	0.205±0.013
After one year of storage		
NE10%	142.1±0.7	0.101±0.016
NE10%-FA	158.3±2.7	0.079±0.001
NE20%	176.0±4.3	0.108±0.019
NE20%-FA	180.9±2.4	0.084±0.019

* insufficient physicochemical stability –gelation was observed within 3 months of storage

To assess the potential stability of the developed nanocarriers, the zeta potential, a measure of the droplet/particle surface charge was determined. In general, positively or negatively charged particles with an absolute zeta potential greater than 30 mV are not considered to be prone to aggregation and agglomeration due to electrostatic repulsive forces (9, 15). The absolute ZP of all samples tested was above 30 mV, with no differences between the NEs and NLCs, indicating that the presence of solid lipid had no effect on the surface charge of the particles. Particularly, it appears that the zeta potential is largely determined by the surfactants used to stabilize the systems (mainly negatively charged phospholipids from egg lecithin). Interestingly, no remarkable differences in pH were observed for the different nanocarrier types with different lipid content. The pH of NLC with 10% and 20% oil phase was slightly lower than that of the corresponding NEs, but the observed difference is very small (< 0.2 pH units) and not relevant for skin tolerance (pH was 4.58 to 4.80, which is considered acceptable for skin application). However, as shown in Table IV, variations in lipid content significantly affected the

Table IV Changes in relevant physicochemical parameters (zeta potential, pH, electrical conductivity) of developed lipid nanocarriers during one year of storage at room temperature (25±2°C)

Tabela IV Promene relevantnih fizičkohemijskih parametara (zeta potencijal, pH, električna provodljivost) ispitivanih lipidnih nanonosača tokom godinu dana čuvanja na sobnoj temperaturi (25±2°C)

Formulation	Zeta potential (mV)	pH	Electrical conductivity (μS/cm)
Initially			
NE10%	-42.0±0.3	4.80±0.00	215.33±1.15
NE10%-FA	-36.0±0.3	4.73±0.01	247.00±1.73
NE20%	-36.1±1.5	4.72±0.01	302.33±3.21
NE20%-FA	-37.5±0.6	4.74±0.01	269.67±3.05
NLC10%*	-43.0±0.5	4.66±0.02	211.70±3.21
NLC10%-FA*	-39.6±0.4	4.44±0.01	187.00±4.93
NLC20%*	-42.7±0.4	4.58±0.01	321.00±13.2
NLC20%-FA*	-41.5±0.8	4.40±0.01	300.80±8.30
After one year of storage			
NE10%	-36.0±0.9	4.14±0.01	284.00±3.00
NE10%-FA	-36.1±0.0	4.06±0.08	308.33±2.31
NE20%	-32.1±0.1	4.16±0.01	319.67±2.08
NE20%-FA	-33.8±1.1	4.26±0.02	330.33±2.08

* insufficient physicochemical stability – gelation was observed within 3 months of storage.

electrical conductivity values of the two lipid nanocarriers developed. Higher lipid phase content (20%) was associated with a significant increase in electrical conductivity values (Table IV), likely due to higher levels of egg lecithin and charged phospholipids. Compared with the corresponding placebos, the incorporation of FA was found to have no significant effect on the monitored parameters of either nanocarrier type, including droplet/particle size, zeta potential, pH, and electrical conductivity, supporting the assumption that FA is predominantly localized in the lipid core.

In vitro release and permeation study

Although *in vivo* bioavailability cannot be directly predicted, an *in vitro* release testing using artificial polycarbonate membranes was conducted to evaluate whether and to what extent the structural features of the developed nanocarriers affect the release of FA, and to gain insight into the factors that may play a role in FA skin release, compared to the marketed reference cream formulation (Sinoderm®, Galenika AD, Serbia). The obtained cumulative release profiles of FA from the developed lipid nanocarriers and the reference cream are shown in Figure 2. The NLC formulation with 20% lipid phase (NLC20%- FA) was excluded from further studies due to stability issues.,

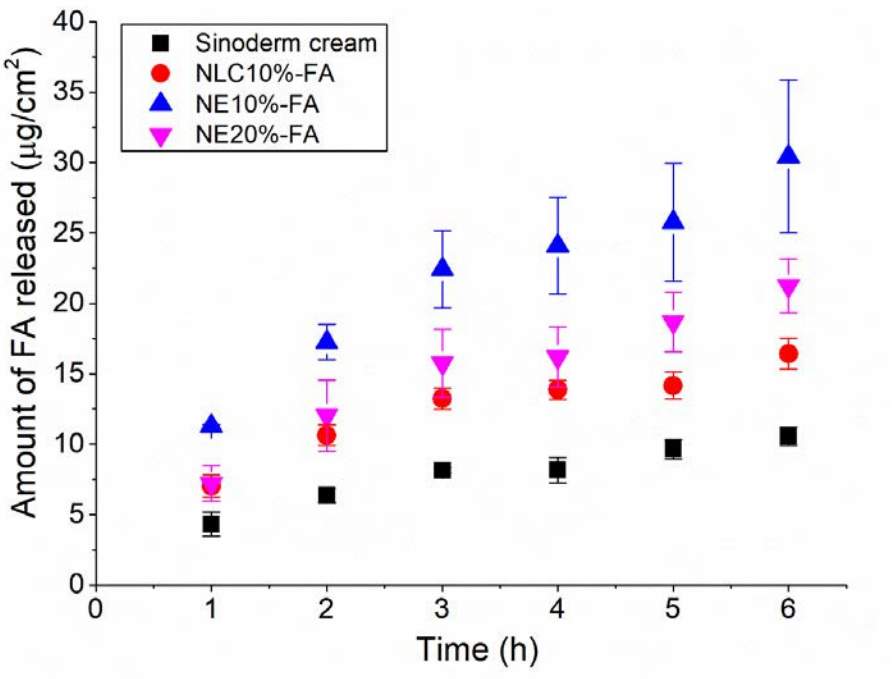

Figure 2. Cumulative release profiles of fluocinolone acetonide (FA) from the tested formulations determined using synthetic polycarbonate membranes (0.1 μm)

Slika 2. Kumulativni profili oslobađanja fluocinolonacetonida (FA) iz ispitivanih formulacija određeni korišćenjem sintetskih polikarbonatnih membrana (0.1 μm)

As shown in Figure 2, it is evident that the novel lipid nanocarriers significantly increased the release rate and the amount of FA released at the end of the test compared to the reference formulation. Considering the significant differences in consistency behavior (the developed nanoformulations are liquid, while the reference sample has a semi-solid consistency), this result was quite expected. In other words, it appears that the diffusion of FA through the semi-solid matrix of the reference sample is the rate-limiting step for release. Interestingly, the *in vitro* release of FA decreased significantly when the lipid phase content was increased, probably due to the longer residence time of FA in a larger amount of nanodroplets (thus, NE20%-FA was excluded from further studies). Notwithstanding, the *in vitro* release of NLC10%-FA was significantly lower compared to NE10%-FA and characterized by a biphasic release pattern – relatively rapid release at the beginning of the assay (between the 1st and 3rd hour), without significant changes between the 3rd and 4th hour, followed by a slight increase from the 5th hour. According to Pradhan and co-authors (4), this initial explosive release may increase the FA penetration into the skin, whereas the sustained release of FA may occur over a longer period of time. The relatively slow and sustained release of FA from NLC10%-FA is due to the specific structure of NLC, i.e., the crystalline matrix, which limits the diffusion of FA (20).

Having in mind that *in vitro* release assays using artificial polycarbonate membranes are not able to mimic the multifaceted interactions between the formulation and the skin, the *in vitro* permeation study was also performed by more biologically relevant membranes, such as the heat-separated porcine epidermis. The *in vitro* permeation profiles of FA from the developed NE (NE10%-FA) and NLC (NLC10%-FA) and the reference cream formulation are shown in Figure 3. The obtained *in vitro* permeation profiles show that the developed lipid nanocarriers with a 10% lipid phase significantly improved the amount of FA permeating through the epidermis compared to the reference sample. Based on the total permeated amount of FA, the formulations studied could be graded as follows: Sinoderm® cream<NLC10%-FA<NE10%-FA. The *in vitro* permeation results were in accordance with the outcomes of the *in vitro* release study. More precisely, although there is some difference (~30 nm) in droplet/particle size between NE10%-FA and NLC10%-FA, this is generally small and cannot be considered relevant to the observed differences in *in vitro* permeation profiles. Therefore, better release is considered to be the main factor influencing FA permeation through the epidermis of the pig ear. However, it should be noted that the literature often describes the superiority of NLCs over NEs in terms of dermal drug delivery, e.g., (9, 21, 22), which is due to the solid lipid in the structure and the better occlusive effect on the skin. The reason for the discrepancy in our results could be the presence of oleic acid, a well-known penetration enhancer, and the specific internal structure of the developed lipid nanocarriers, which requires further investigation.

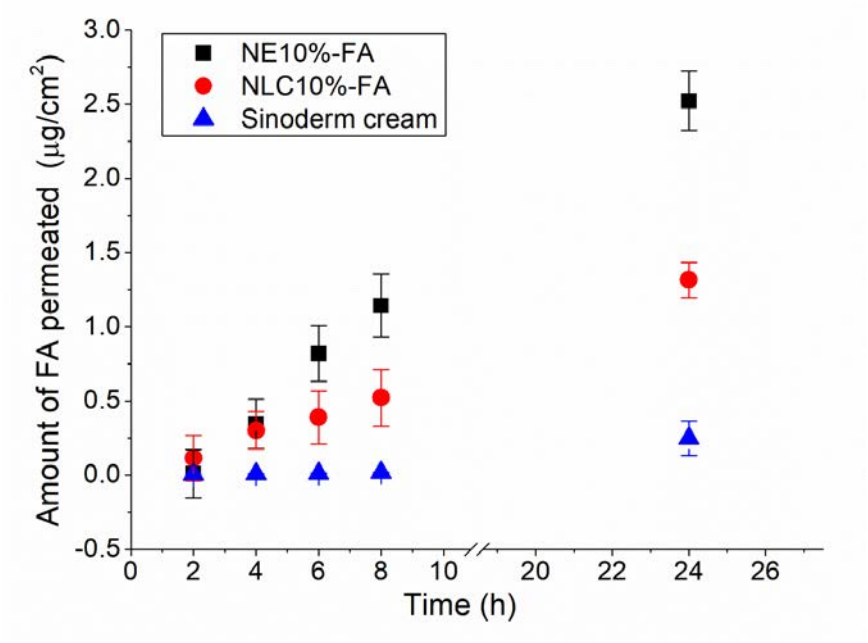

Figure 3. **Cumulative permeation profiles of fluocinolone acetonide (FA) from the tested formulations determined using porcine ear epidermis as the membrane**

Slika 3. **Kumulativni profili permeacije fluocinolonacetonida (FA) iz ispitivanih formulacija određeni korišćenjem epidermisa kože uha svinje kao membrane**

Long-term stability study

The physicochemical stability of the developed lipid nanocarriers was regularly examined during one year of storage at room temperature for changes in the following relevant physicochemical parameters: droplet/particle size and size distribution, pH, zeta potential and electrical conductivity. All placebo and FA-loaded NEs tested were liquid, milky-white with bluish coloration (creaming and phase separation were not detected). In contrast, the NLC formulation with a 10% lipid phase (NLC10%- FA) began to gel shortly after preparation, clearly indicating limited stability of this system (particle size measurement was not possible). During a single year of storage at room temperature, negligible changes in droplet size and PDI values were observed for both placebo and FA-loaded NEs with 10% and 20% lipid phase (Table III). The PDI of the developed NEs remained < 0.15, demonstrating the absence of droplet aggregation and coalescence during the storage period. Moreover, all tested NEs still exhibited high negative zeta potential values (ranging from -32 to -36 mV), indicating high surface charge and droplet repulsion preventing coalescence. On the other hand, a marked decrease in pH was observed, probably due to the hydrolytic/oxidative degradation of phospholipids (23). The decrease in pH during storage of NEs has been frequently reported in the literature and usually requires a change in the antioxidant used. However, all pH values were still within the acceptable range for skin application. In addition, as shown in Table IV, the

electrical conductivity values of all tested NEs increased during a single year of storage at room temperature, which is consistent with the observed pH decrease. However, the changes in this parameter cannot be a reliable indicator of nanoemulsion instability because no direct correlation was found between the increase in electrical conductivity and emulsion system instability (24).

Conclusion

Despite the numerous challenges in formulation development, FA-loaded NEs with 10% and 20% oil phase, satisfactory physicochemical properties and long-term stability at room temperature 25 ± 2°C were successfully developed. Although the developed NLCs initially exhibited satisfactory physicochemical properties, gelation occurred within three months of storage, clearly indicating that these systems were not sufficiently stable. Therefore, further formulation tests are needed to improve the physicochemical stability of NLCs during prolonged storage.

The results of *in vitro* biopharmaceutical evaluation using vertical diffusion cells equipped with the artificial polycarbonate membrane or porcine ear epidermis revealed that the developed NE with 10% oil provides better *in vitro* release and permeation of FA compared to the reference cream, Sinoderm®. Therefore, the NE stabilized with a mixture of lecithin and polysorbate 80, with a 10% oil phase containing a mixture of medium-chain triglycerides and oleic acid, is a promising strategy for improved FA delivery in inflammatory skin diseases, allowing easy application, especially on larger skin surfaces and in hairy regions, and thus improving patient adherence. However, the risk of systemic absorption and resulting adverse effects require further detail investigation.

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Biokompatibilni lipidni nanonosači za poboljšanu isporuku fluocinolonacetonida u kožu: fizičko-hemijske osobine i *in vitro* učinak

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Kratak sadržaj

Kako bi se poboljšala topikalna isporuka kortikosteroida u inflamatorne lezije kože i istovremeno smanjila učestalost neželjenih efekata, posebna pažnja je usmerena ka razvoju lipidnih nanonosača. Stoga, cilj ovog rada je bio razvoj biokompatibilnih nanoemulzija (NEs) i nanostrukturiranih lipidnih nosača (NLCs) kao nosača za fluocinolonacetonid (FA) pažljivom optimizacijom formulacionih i procesnih parametara. Nakon analize relevantnih fizičko-hemijskih parametara i studije stabilnosti, *in vitro* ispitivanje oslobađanja i permeacije je sprovedeno kako bi se dobio uvid u to da li razvijeni nanonosači utiču na penetraciju FA u/kroz kožu, u poređenju sa konvencionalnim referentnim preparatom (Sinoderm® krem). Uspešno su razvijene NEs zadovoljavajućih fizičko-hemijskih osobina (veličina kapi<200 nm, PDI<0,2, ZP>|-30| mV, pH~4,75) i dugoročne stabilnosti. Iako su inicijalno posedovali zadovoljavajuće karakteristike, NLCs su gelirali tokom tri meseca čuvanja, što ukazuje na potrebu za daljim radom na razvoju formulacije, u cilju rešavanja problema ograničene stabilnosti ovih sistema. Nalazi *in vitro* ispitivanja oslobađanja/permeacije upućuju na činjenicu da razvijeni lipidni nanonosači (prevashodno NEs) obezbeđuju bolju isporuku FA u/kroz kožu u poređenju sa Sinoderm® kremom. Nanoemulzije bazirane na lecitinu sa 10% uljane faze (smeša triglicerida srednje dužine lanaca i oleinske kiseline 3:1) predstavljaju obećavajuću strategiju za poboljšanu isporuku FA u inflamatorne promene na koži, omogućavajući laku primenu, posebno na većim površinama i kosmatim delovima tela.

Ključne reči: fluocinolonacetonid, nanoemulzije, nanostrukturirani lipidni nosači, dermalna isporuka, veličina kapi