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A novel formulation strategy for skin occlusion: Semi-solid lipid nanoparticles

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ABSTRACT: Lipid nanoparticles (SLNs and NLCs) have possessed many advantages as versatile and innovative carriers to achieve better quality and highly effective skin-care cosmeceuticals. While they protect the water content of the epidermis by increasing skin occlusion, maintain the smoothness and elasticity of the skin, enhance the efficacy of the active substance by penetrating into the deep layers of the skin. Semi-solid lipid nanoparticles, which are the latest generation of lipid nanoparticles containing high amount of lipids compared to SLNs and NLCs are promising systems as nano-cosmeceuticals due to their semi-solid consistency and colloidal particle size. In this study, semi-solid lipid nanoparticles in NLC form were obtained by using different vegetable oils that possess positive effects in the dermal application. Then, the main *in vitro* physicochemical characteristics of the formulations that affect skin hydration like size, surface charge, and occlusive properties were investigated. Overall results suggested that, while vegetable oil loaded semi-solid lipid nanoparticles were obtained, colloidal size and semi-solid consistency could be maintained. On the other hand, all formulations had acceptable firmness values for customer use despite the high lipid content of the system, and enhanced skin hydration with the help of both vegetable oils and lipid nanoparticles were achieved.

KEYWORDS: Semi-solid lipid nanoparticles; nano-cosmeceuticals; vegetable oil; skin occlusion.

1. INTRODUCTION

Besides its physiological functions, the young and healthy appearance of the skin also has a positive effect on our social behaviour and adequate skin hydration is required to maintain skin health. Stratum corneum, the uppermost layer of the skin, acts as the principal layer between the body and the external environment. Two main components of the stratum corneum, the corneocytes, and extracellular lipids serve the brick and mortar structure and contribute to the barrier function of the stratum corneum [1-2]. Thus, the complex nature of the stratum corneum limits the penetration of foreign compounds such as chemicals or infectious agents into the skin layer while preventing loss of water from the body and keeping the skin moisturized [3].

Although skin aging is a natural process in which both intrinsic and extrinsic factors come into play, UV rays have an important role in skin aging. Structural changes are observed in the skin over time and total lipid content and hygroscopic agents called natural moisturizing factors (NMF) or ceramide levels as well are significantly depleted which causes water loss in the skin [4]. Changes that occur as a result of skin aging can be observed in all layers of the skin. Loss of elasticity, wrinkles, dry and thin skin, as well as spotting, are the most basic symptoms that can be associated with skin aging [5]. Using routine cosmeceuticals in daily life is one of the most preferred approaches to slow down the sign of skin aging. The occlusive effect is essential for cosmeceutical formulations as it promotes to increase skin hydration and heals dryness of the skin, reduces fine lines and wrinkles caused by dryness, and makes the skin smooth and soft. Hydrocarbon-based petrolatum, mineral oil, and paraffin are classical occlusives that are the most frequently used in a cosmetic product. But they have poor customer acceptance because of their greasy, sticky, and unwashable nature.

Cosmeceuticals or dermocosmetics are basically defined as functional cosmetics and typical cosmeceutical product involves active ingredients that display skin positive effects and are delivered by a vehicle like cream or lotion [6-7]. Cosmeceuticals have gained many new perspectives with the advances in nanotechnology and since the first approved nanocosmeceutical product was launched by Dior in 1986; the

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classic cosmetic preparations have been replaced by nanotechnology-based cosmeceuticals. Although there are various new nanocarriers for the delivery of cosmetic active molecules, lipid-based nanosystems are at the forefront due to their advantages in dermal applications, and today many lipid-based nanocosmeceutical like liposome, nanoemulsion, and lipid nanoparticles is available on the global market. The physiological lipid composition of the lipid-based nanosystems makes them valuable and provides them to acquire biocompatible and biodegradable properties. Among the lipid-based nanosystem, in particular, lipid nanoparticles seem to be highly promising as cosmeceuticals. Lipid nanoparticles are made of typically lipids or lipid mixtures like triglycerides, cholesterol, or waxes that are already present in our cells and the stratum corneum layer. Lipids are solid at both room and body temperature and stabilized by suitable surfactants to form spherical lipid matrix. They are called solid lipid nanoparticles (SLN) if they obtained using only solid lipids, and nanostructured lipid carriers (NLC) if they consist of solid lipid - liquid lipid mixtures. The unique feature of lipid nanoparticles as cosmeceuticals is the ability to improve skin occlusion. The occlusion is responsible for the reduction of transepidermal water loss (TEWL), and smoothing effect on wrinkles as well. Moreover, easy scale-up procedures and using relatively low-cost raw materials make lipid nanoparticles more preferable [8-9]. But, one of the most important disadvantages is that they are obtained in the form of aqueous dispersions whereas the main requirement for applying nano-carriers to the skin is that they need to be homogeneously incorporated into semi-solid systems such as cream, lotions, or hydrogels. The second system to carry nanocarriers is necessary both for ease of application and to obtain attractive products for the customer [10-11]. Aforementioned obstacle that increases the cost of the product and also causes loss of time, has been eliminated by the development of semi-solid lipid nanoparticles. Semi-solid lipid nanoparticles are the latest generation of lipid nanoparticles with semi-solid consistency instead of aqueous nanoparticle dispersion. The semi-solid form is associated with a high lipid content. On the other hand, since semi-solid consistency can be obtained in one-step procedure, it provides ease of production and there is no need to use extra excipients. The final product can be applied directly on the skin without any further process. It has also been reported that the semi-solid system preserves both colloidal size and viscoelastic properties, but a gel-like structure can be obtained despite the high lipid content [12-13]. Semi-solid lipid nanoparticles can be prepared in SLN or NLC form depending on the use of solid lipid or both solid lipid and liquid lipid mixture.

Moreover, semi-solid lipid nanoparticles are fairly new systems, and they exhibit many advantages over lipid nanoparticle dispersions. Although very limited studies have been published in the pharmaceutical field, it has not yet focused on the cosmeceutical application of semi-solid lipid nanoparticles. The major purpose of this work is to develop novel nanocosmeceuticals by combining the antiaging effect of vegetable oils with the advantages of lipid nanoparticles and to investigate the profits of selecting natural lipids as structural components of semi-solid lipid nanoparticles for cosmeceutical application.

2. RESULTS

This study was designed to investigate the possible effect of active vegetable oils on the physicochemical features of the semi-solid lipid nanoparticles which will be used as nano-cosmeceuticals. Herein, the first group of semi-solid lipid nanoparticles was produced as SLN to determine the solid lipid composition. Semi-solid SLNs were formed using 30% (w/w) solid lipid. Precirol® ATO 5, which is a monoglyceride, consists of esters of palmitic, and stearic acids; stearic acid which is a fatty acid; and the binary mixture of the solid lipids were used to prepare Semi-solid SLNs. Poloxamer 188 was used as a stabilizer at a concentration of 10% (w/w). It is reported that while stearic acid is the most used solid lipid for preparing lipid nanoparticles, Poloxamer 188 is the most widely preferred non-ionic stabilizing agent [14]. The composition of semi-solid lipid nanoparticle formulations was given in Table 3. Hot homogenization using high energy was used as a production method, consisting of high shear homogenization step followed by ultrasonication to obtain the final semisolid product. The primary reason to choose the hot homogenization method is that there is no need to use organic solvents. While the formation of the stable emulsion is ensured by the high shear homogenization step, which is a simple and reproducible method, particle size can be reduced by ultrasonication.

When dynamic light scattering (DLS) results of the Semi-solid SLNs were examined, it can be seen that the mean particle size of stearic acid based SemiSLN-1 was 497 nm with a PDI value of 0.311 whereas Precirol® ATO 5 based formulation (SemiSLN-2) was approximately 383 nm in size with a PDI of 0.414. Interestingly, the obtained values of particle size and PDI of SemiSLN-3 composed of 1:1 stearic acid and Precirol® ATO 5 mixture were found to be 260 nm and 0.215 respectively. The results obtained from the binary solid lipid mixtures offered optimum particle size and size distribution compared to using these lipids alone. This may

be possible because the binary lipid matrix causes deformation of the perfect crystalline lattice and is facilitated to break down into small particles. On the other hand, various factors such as the change in the melting point of the lipid mixture and the viscosity of the dispersed phase could affect the particle size of the semi-solid SLNs [15-16]. The results also suggested that the binary system had a positive effect on PDI, which is an indication of homogeneity of the particle distribution. Clearly, the lowest PDI value means monodisperse particle distribution [17]. For Semi-solid SLNs, highly negative zeta potential values were measured in the range of -33.7 – 22.8 mV as reported in Table 1. Also, binary mixtures of two solid lipids appeared to decrease the zeta potential of the nanoparticles as compared to a single solid lipid was used.

Table 1. DLS results of the semi-solid lipid nanoparticle formulations.

	Particle size (nm)±SD	PDI±SD	Zeta Potential (mV)±SD
SemiSLN-1	496.9±1.273	0.311±0.003	-33.7±0.69
SemiSLN-2	382.8±5.411	0.414±0.018	-23.3±0.46
SemiSLN-3	260.9±3.120	0.215±0.012	-22.8±0.14
SemiNLC-1	247.9±0.212	0.439±0.040	-36.6±1.36
SemiNLC-2	218.0±2.307	0.294±0.020	-37.6±0.75
SemiNLC-3	208.3±2.400	0.367±0.015	-37.4±0.78
SemiNLC-4	302.6±2.475	0.441±0.003	-27.0±0.49
SemiNLC-5	272.9±2.598	0.372±0.027	-33.8±1.05
SemiNLC-6	232.3±3.225	0.498±0.018	-31.5±0.50
SemiNLC-7	294.9±4.618	0.471±0.016	-33.4±0.66
SemiNLC-8	244.0±4.339	0.345±0.016	-37.8±0.55
SemiNLC-9	209.4±5.501	0.293±0.009	-40.5±0.81
SemiNLC-10	418.1±3.041	0.485±0.016	-30.2±0.87
SemiNLC-11	244.3±2.957	0.385±0.044	-32.4±0.20
SemiNLC-12	214.4±2.007	0.360±0.016	-34.6±1.15
SemiNLC-13	429.3±2.427	0.478±0.019	-33.5±0.44
SemiNLC-14	308.7±4.808	0.454±0.037	-33.5±0.62
SemiNLC-15	227.0±0.400	0.437±0.003	-37.4±0.56

Following the characterization of the Semi-solid SLNs, SemiSLN-3 was chosen due to the smallest particle size and PDI value. Then, 30%, 40%, or 50% of the solid lipid mixture was replaced with liquid lipids to produce Semi-solid NLC formulations. For this purpose, pure argan oil, coconut oil, and avocado oil used as vegetable oil. Also, synthetic oils Miglyol® 812 which is the esters of saturated coconut and palm kernel oil derived caprylic and capric fatty acids and glycerine; or pure oleic acid which is the principal fatty acid found in olive oil were used as liquid lipid. When all characterization results were evaluated, it is possible to obtain Semi-solid NLCs in the nanometer range with all liquid lipids successfully. Vegetable oils derivate from a plant source like vegetables, fruits or seeds are well known for their health benefits and they have been safely used on the skin since ancient times. Edible vegetable oils obtained from different sources have their own composition but generally, they are rich in triglycerides, fatty acids, and other minor components like tocopherols, phenolic compounds, carotenes, squalene and essential fatty acids as well. Vegetable oils also defined as fixed oils are generally liquid at room temperature. They play a major role in cosmetic products due to their high palmitic, stearic, oleic, and linoleic acid contents and the most frequently used vegetable oils in cosmetics are argan oil, coconut oil, olive oil, soybean oil, avocado oil, and palm oil [18]. Vegetable oils are most often used in cosmetics to provide occlusive, emollient, moisturizing and grooming effects, acting as solvents and vehicles to carry other agents as well. When vegetable oils are used as a liquid lipid to produce NLC, kind of vegetable oil loaded lipid nanoparticles could be obtained. On the other hand, synthetic oils Miglyol® 812 and oleic acid are among the most preferred liquid lipids in the structure of NLCs developed as drug delivery systems.

Semi-solid NLC formulations were successfully produced with average particle size ranging from 208 and 429 nm and PDI values were found smaller than 0.498 as seen in Table 1. It has been observed that the particle size of Semi-solid NLCs was significantly decreased with the increase in the concentration of liquid lipid. The structure of the lipid matrix is one of the most important factors on particle size. As previously stated in the literature, the increase in the amount of vegetable oil is believed to cause the reduction of the particle size due to the fact that the liquid lipid can be dispersed more easily in the aqueous phase [19-20].

Also, the presence of liquid lipid in addition to binary solid lipid mixture significantly reduced particle size by reducing the lipid phase viscosity. On the other hand, adding liquid lipid to the system was found to be effective in enhancing zeta potential values, and an increased surface charge density with all Semi-solid NLC formulations was achieved; whereas it caused an increase in particle size distribution in comparison to SemiSLN-3.

While the pH of the skin surface has been reported in the acidic range between 5 and 7, it is observed that pH value rises to the neutral pH towards the dermis as all other tissues [21-22]. The acidic nature of the stratum corneum is associated with free fatty acids in the sebum, microbial metabolites, and lactic acid secretion and it provides to keep the skin in good condition. A calibrated digital pH meter was used to measure the pH values of semi-solid lipid nanoparticles and the pH measurement results of all formulations were found between 6.2 ± 0.04 and 6.31 ± 0.01, compatible with the physiological pH of the skin (Table 2). When skin surface pH and targeting of lipid nanoparticles to deep dermal tissue are evaluated together, it is considered that the pH of the all formulations will eliminate the possibility of skin reactions and irritations in long-term cosmeceutical applications.

The viscosity and the flow behavior of the topical semisolid formulations are important parameters due to the fact that it must be applied to the outer layer of skin and it may influence the spreadability of the formulation. The viscosity measurements of the formulations were performed by a Brookfield viscometer with a T96 spindle at 20 rpm and the results were presented in Table 2. When stearic acid and Precirol® ATO 5 based semi-solid SLNs were compared; a higher viscosity value was obtained with Precirol® ATO 5 and it was observed that Semi-solid SLN with a binary mixture of lipids exhibited the lowest viscosity value. It is obviously seen that when different vegetable oils were added as a liquid lipid to the binary lipid mixture system, the viscosity of the Semi-solid NLCs decreased depending on the liquid lipid type and concentrations. However, surprisingly, an increase in viscosity was obtained in the presence of 30% concentration of oleic acid. The increase in viscosity despite the addition of liquid lipid may be related to the increase in particle size and PDI value of the formulation with SemiNLC-13 coded formulation containing oleic acid. For Semi-solid NLCs, when the concentration of each vegetable oil was increased from 30% to 50%, a decrease was obtained in the viscosity values of the semi-solid system due to the increase in the concentration of liquid lipid. It could be suggested that the viscosity of the Semi-solid NLCs is significantly influenced by the composition of the vegetable oils. Because the strength of semi-solid could be mainly attributed to the differences in the fatty acid composition of vegetable oils.

Table 2. pH, viscosity and firmness results of the semi-solid lipid nanoparticle formulations.

	pH±SD	Viscosity ($cPx10^3$) ± SD	Firmness (N)±SD
SemiSLN-1	6.26±0.03	146.50±2.51	1.137±0.074
SemiSLN-2	6.17±0.01	263.17±2.04	2.695±0.238
SemiSLN-3	6.31±0.06	125.0±2.97	1.397±0.130
SemiNLC-1	6.22±0.02	73.0±1.79	0.810±0.048
SemiNLC-2	6.31±0.01	34.67±1.21	0.613±0.085
SemiNLC-3	6.20±0.02	27.33±1.03	0.166±0.006
SemiNLC-4	6.24±0.02	103.17±2.14	0.529±0.032
SemiNLC-5	6.15±0.04	76.83±2.04	0.319±0.002
SemiNLC-6	6.17±0.01	10.50±0.55	0.053±0.004
SemiNLC-7	6.22±0.01	50.33±0.82	1.519±0.041
SemiNLC-8	6.29±0.01	36.05±1.79	0.368±0.070
SemiNLC-9	6.15±0.01	19.50±1.38	0.313±0.008
SemiNLC-10	6.12±0.04	38.00±2.28	0.169±0.006
SemiNLC-11	6.18±0.01	22.33±0.82	0.151±0.007
SemiNLC-12	6.16±0.02	13.83±0.75	0.063±0.003
SemiNLC-13	6.13±0.01	216.67±3.20	1.453±0.118
SemiNLC-14	6.20±0.03	128.33±1.21	0.104±0.005
SemiNLC-15	6.24±0.02	100.5±2.51	0.036±0.002

Another important feature of semi-solids as well as their viscosity is the mechanical properties for estimating real-life usage and also patient or costomer acceptability [23]. Texture Profile Analysis (TPA), based on the application of controlled force to a product and measuring its response over time, is a simple and effective method used to characterize the mechanical properties of semi-solid drug formulations and cosmetic

products as well. Various parameters such as firmness, elasticity, adhesiveness, compressibility, spreadability, or cohesiveness can be examined by TPA analysis and these parameters provide information about the behaviour of semi-solids during both production and application. Firmness or in other words, hardness is one of the most commonly evaluated mechanical parameters of semi-solids where it refers to the applicability of the formulation to the skin surface and defines the maximum force required to achieve a certain deformation. TA.XT Plus Texture Analyzer was utilized in compression mode with a 5 kg load cell and a trigger force of 0.05 N to measure the maximum peak force versus time andto calculate the firmness value of the semi-solid lipid nanoparticle formulations. It should be noted that a lower hardness value is desired in order to remove the semi-solid product from the container and to spread it to the skin surface easily [24-25]. As presented in Table 2; SemiSLN-2 had the highest firmness value of 2.695 N, while the lowest firmness value of 1.137 N was measured for semiSLN-1. However, binary lipid mixture based SemiSLN-3 had a 1.3 N firmness value although it had a lower viscosity value than SemiSLN-1. It can be indicated that the presence of two different solid lipid mixtures affected the consistency of the system. On the other hand, the firmness values of semi-solid NLCs were in the range of 0.036±0.002 and 1.519±0.041 N. It can be seen that the consistency, as well as the firmness of the semi-solid structure, was strongly influenced by the type and amount of vegetable oil. Apart from the Semi-solid NLCs prepared with the lowest concentration of coconut oil and oleic acid; it can be said that in all semi-solid NLCs, lower firmness values were obtained than SemiSLN-3. Additionally, in all Semi-solid NLCs, a decrease in firmness values was observed due to the increase in the concentrations of vegetable oils used as liquid lipids. As a consequence, NLC structure as well as the presence of vegetable oil could improve the textural properties of the semisolid formulation in terms of firmness.

Lipid nanoparticles have cosmeceutical advantages when applied to the skin, even if they do not contain any active molecules. Solid particles form an adhesive lipid film layer on the skin surface after application and an increase in occlusive effect is provided by means of the lipid film layer. Thus, the water content of stratum corneum is increased, TEWL is reduced, and an increase in skin hydration can be achieved. *In vitro/in vivo* studies have shown that semi-solid formulations containing SLN have significantly increased skin hydration compared to conventional O/W cream formulations [26-28]. On the other hand, with increased skin hydration, it may be easier for active substances to reach the deep skin tissue. This means skin permeation may be enhanced and essential fatty acids could reach the dermal cells by lipid nanoparticle composition. Herein, the contribution of vegetable oils from different sources to the occlusive effect of semi-solid lipid nanoparticles has been investigated by *in vitro* occlusion test developed by de Vringer [29]. Using vegetable oil, which has potent skin hydration properties and physiological effects when applied to the skin, to construct semi-solid lipid nanoparticles; is a promising technique for cosmeceuticals due to the ability to target essential fatty acids into the cells with NLC structure. Argan oil, avocado oil, coconut oil as natural vegetable oils; Miglyol® 812 and oleic acid which are synthetic oils of vegetable origin, were used as liquid lipids to form semi-solid NLCs. The lipid content of Avocado oil is mainly of monounsaturated fatty acids and due to the high concentration of polyphenols and vitamin E, it possesses antioxidant properties [30]. Argan oil is rich in linoleic acid which is an essential fatty acid [31]. On the other hand, coconut oil is a traditional skin moisturizer for centuries and it is the highest natural source of lauric acid that imparts antibacterial properties to coconut oil [32]. *In vitro* occlusion test was performed during a period of 24 hours to calculate occlusion factor for 6th, 12th and 24th hours as given in Figure 1. Also, the relationship between particle size and occlusion factor values at the end of the 24th hour is schematized in Figure 2.

In the initial hours of the occlusion test, (6th hour) the highest values of the F for semi-solid SLNs were obtained with SemiSLN-3 which contains two solid lipid mixture in comparison with SemiSLN-1 and semiSLN-2. Particle size, lipid concentration, and crystallinity are among the most efficient parameters on the occlusion and as a result of the decrement of particle size with binary lipid matrix, a significant increase in occlusive effect was achieved in all time intervals for semi-solid SLN formulations. It has been stated that the reduction in particle size increased the number of particles and increased the surface area, thus contributing to the occlusive effect by increasing the adhesive properties of the particles [33]. However, a similar effect of particle size in semi-solid NLC formulations could not be observed due to the change in the crystal structure of the lipid particle system. For example, in semi-solid NLCs prepared with argan oil, although the particle size decreased with the increase in argan oil concentration, the occlusive effect of SemiNLC-1; SemiNLC-2 and SemiNLC-3 also decreased in all time intervals. Occlusion profile of semi-solid NLCs behaved similar to argan oil in the presence of avocado oil, coconut oil and Miglyol® 812 except oleic acid. On the other hand, at the end of the 6th hour, the lowest occlusion percentage was found only 30% (SemiNLC-8) with Semi-solid NLCs prepared with coconut oil. Depending on the increase in coconut oil concentration, the F value was found to

be 40% for SemiNLC-9. Besides, Semi-solid NLCs composed of Miglyol® 812 displayed F values between 35 – 40%. Although, Miglyol® 812 is one of the most commonly used liquid lipids in the structure of NLCs that is applied as a topical drug delivery system, and in various cosmetic products as well, the obtained occlusive effect is not as sufficient as natural vegetable oils. However, it is interesting to note that SemiNLC-3 with 50% argan oil and SemiNLC-4 with %30 avocado oil presented a very close occlusive effect at the end of the 6th hours. From the results presented in Figure 2, it could be seen that, after 24 h, SemiSLN-3 presented the highest F Value of 75%. On the other hand, despite the low solid lipid ratio of SemiNLC-1 with 30% argan oil and SemiNLC-4 with %30 avocado oil presented very close results to SemiSLN-3 at the end of 24th hour. It is clear that the use of natural vegetable oil for preparing semi-solid NLCs has a significant effect on the occlusion. The occlusion factor of SemiNLC-15 reached to 73.5% after 24 h. Besides, an increase in occlusion factors was obtained depending on time for each individually liquid lipid. It is previously reported that in general, SLN has higher occlusion factor compared to NLC with the same lipid content [34]. When the occlusive effect versus time graphs of semi-solid NLCs in Figure 2 was examined; it could be concluded that the closest results to SemiSLN-3 could be reached by the semisolid NLC formulation containing three different concentration of argan oil (SemiNLC-1; SemiNLC-2; SemiNLC-3), whereas coconut oil had the least contribution to the occlusive effect of the semisolid system. Notably; lower occlusion factor values were attained with semi-solid NLCs containing moderate concentrations of vegetable oils; compared to SemiSLN-3. As a result, the composition and proportions of fatty acids in vegetable oil could determine the occlusive character of semisolid NLCs and it could be possible to obtain similar occlusive effect as SLN formulations.

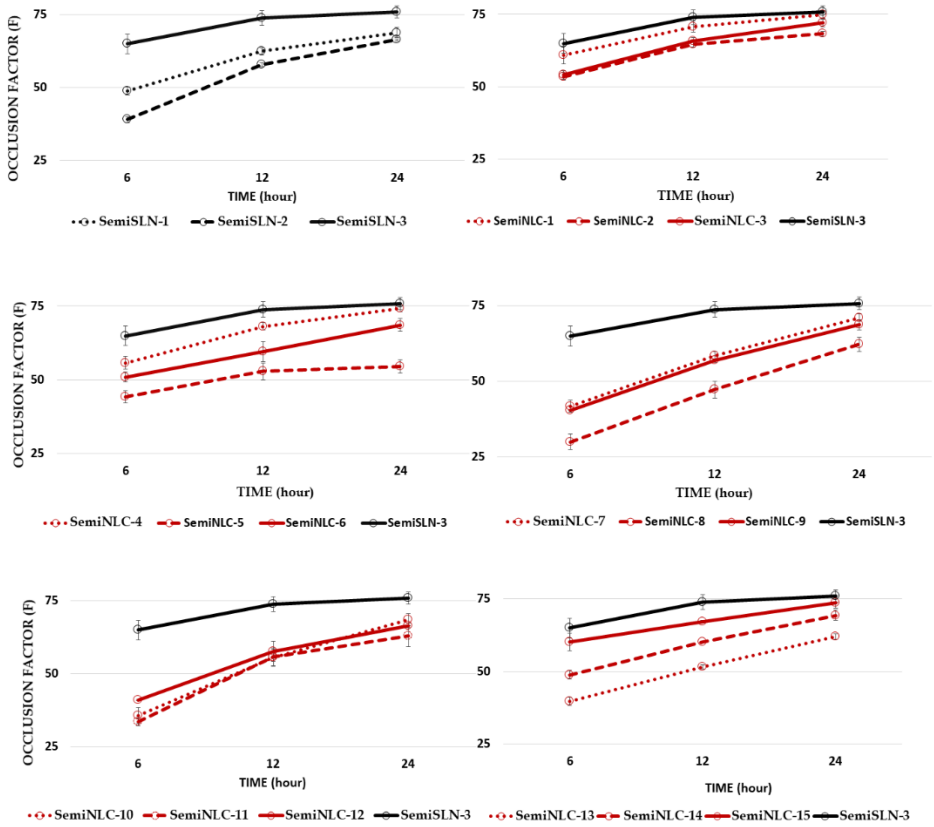

Figure 1. *In vitro* occlusion profile of semisolid SLN and NLC formulations versus time.

3. CONCLUSION

Nanotechnology-based cosmeceuticals offer great superiority for many skin conditions such as photoaging, hyperpigmentation, dryness, or wrinkles. Semi-solid lipid nanoparticles, which are the latest generation of lipid-based nanocarriers, are truly promising system for the skin-care cosmeceutical market considering both their effectiveness, costs, and simple production process. The main advantage of nanocosmeceuticals over conventional cosmetics is that they have particle sizes in the nano range. On the other hand, the use of vegetable oils for cosmetic purposes is quite popular nowadays since they used as skin

moisturizers due to their composition. Herein, the advantages of lipid nanoparticles and vegetable oils were combined in semi-solid NLC structure and semi-solid NLCs containing vegetable oils from different sources have been successfully developed for cosmeceutical application. Using vegetable oil which has potent skin hydration properties and physiological effects when applied to the skin, to construct semi-solid lipid nanoparticles is a promising technique for cosmeceuticals. Another advantage is that it is possible to target essential fatty acids or other components in vegetable oils to skin cells with the NLC structure. In conclusion, when the occlusion properties of both lipid nanoparticles and vegetable oils come together, it could be possible to observe a synergistic effect on skin hydration. Moreover, very limited studies have been published on the drug loaded semil-solid lipid nanoparticles as pharmaceuticals; either cosmeceutical application of semi-solid lipid nanoparticles are not focused yet.

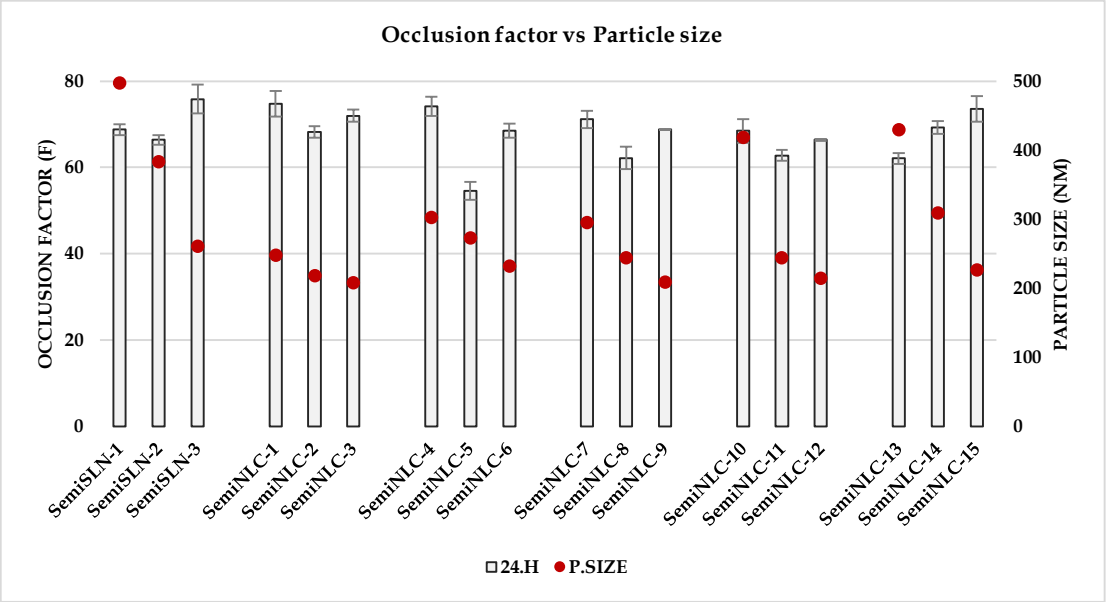

Figure 2. Mean particle size and occlusion factor at the end of 24th hour of semi-solid lipid nanoparticles.

4. MATERIALS AND METHODS

4.1. Materials

Stearic acid, oleic acid; Poloxamer 188 and glycerine were purchased from Sigma–Aldrich, Inc. (Germany) and Precirol® ATO 5 (glyceryl palmitostearate) were kindly provided by Gattefosse (France). Miglyol® 812 was kindly purchased from Caelo (Germany). While avocado oil was obtained from Arifoglu Company (Turkey); coconut oil was provided by Zade-Vital Company (Turkey); argan oil and the preservative Biomix ECO were also gifted from Mecitefendi Company (Turkey). All other chemicals were of analytical reagent grade and MilliQ water was used for all experiments.

4.2. Semi-solid lipid nanoparticle formations

Semi-solid lipid nanoparticles were prepared by hot homogenization method which utilized both high shear homogenization and ultrasonication. Briefly, the required amount of solid lipid or solid lipid vegetable oil mixture was weighed into a glass beaker and then the mixture was heated to 80°C into a water bath. For the aqueous phase, Poloxamer 188 was dissolved into water and then was heated to 80 °C in another glass beaker. A high shear homogenizer (UltraTurrax T18, IKA, Germany) was employed to homogenize both lipid and aqueous phases at 24000 rpm for 5 min. The probe sonicator with a 6 mm diameter (VibraCell 500, Sonics, USA) was used for 5 min to obtain nanosized lipid dispersion at the second step of the production. The obtained hot SLN/NLC dispersion was cooled at room temperature to form a semi-solid structure. Before the formulations were stored at 4°C overnight, 2% glycerine as a humectant and 0.5% Biomix ECO as the

preservative were added and ultrapure water was used to obtain the final quantity of the formulations of 25g and mixed homogenously. The formulation parameters are shown in Table 3.

Table 3. The composition of the semi-solid lipid nanoparticle formulations.

	Stearic acid (g)	Precirol® ATO 5 (g)	Argan oil (g)	Coconut oil (g)	Avocado oil (g)	Myglyol® 812 (g)	Oleic acid (g)
SemiSLN-1	7.5	0	0	0	0	0	0
SemiSLN-2	0	7.5	0	0	0	0	0
SemiSLN-3	3.75	3.75	0	0	0	0	0
SemiNLC-1	2.625	2.625	2.25	0	0	0	0
SemiNLC-2	2.25	2.25	3	0	0	0	0
SemiNLC-3	1.875	1.875	3.75	0	0	0	0
SemiNLC-4	2.625	2.625	0	2.25	0	0	0
SemiNLC-5	2.25	2.25	0	3	0	0	0
SemiNLC-6	1.875	1.875	0	3.75	0	0	0
SemiNLC-7	2.625	2.625	0	0	2.25	0	0
SemiNLC-8	2.25	2.25	0	0	3	0	0
SemiNLC-9	1.875	1.875	0	0	3.75	0	0
SemiNLC-10	2.625	2.625	0	0	0	2.25	0
SemiNLC-11	2.25	2.25	0	0	0	3	0
SemiNLC-12	1.875	1.875	0	0	0	3.75	0
SemiNLC-13	2.625	2.625	0	0	0	0	2.25
SemiNLC-14	2.25	2.25	0	0	0	0	3
SemiNLC-15	1.875	1.875	0	0	0	0	3.75

4.3. Characterization of semi-solid lipid nanoparticle formulations

4.3.1. Particle size, size distribution and the zeta potential measurement

The average particle size and size distribution of semi-solid lipid nanoparticles were measured through photon correlation spectroscopy (PCS) (Dynamic Light Scattering, DLS, Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) at 633 nm wavelength of a scattering angle of 173° at 25°C. Same samples for size analysis were used to determine the zeta potential of the formulations by using Zetasizer Nano ZS. In order to obtain the suitable scattering intensity, all samples were diluted with ultra-purified water and all measurements were done in triplicate.

4.3.2. Viscosity and pH analysis of semi-solid lipid nanoparticle formulations

The determination of pH of the semi-solid lipid nanoparticle formulations was performed by a pH-meter (Mettler Toledo S-20 K, Switzerland) in triplicate at room temperature. For this purpose, all formulations were diluted 10 times with ultrapure water and the glass pH electrode was directly dipped into each sample (n=3). The dynamic viscosity of the formulations was examined by a rotational viscosimeter (Brookfield SD II / Brookfield DV II, USA) using a T96 spindle at 20rpm. The temperature was set at 25 ± 1° C during analysis (n = 9). All pH and dynamic viscosity results were expressed as mean ± standard deviation.

4.3.3. Mechanical characterization of semi-solid lipid nanoparticle formulations

The textural attributes of firmness was determined in the compression mode by a TA.XT Plus Texture Analyzer (Stable Micro Systems, UK) with a 5kg load cell and a trigger force of 0.05 N. For the analysis, a 25g sample was placed in a 2 cm diameter glass beaker without any air bubbles and a cylindrical perspex probe with a diameter of 10 mm was immersed into the sample with a defined velocity and force. The maximum force was calculated from the force versus distance graph and the measurements were performed in triplicate at 25 °C.

4.3.4. Determination of the in vitro occlusion factor (F)

The *in vitro* occlusion test was adapted from the procedure reported by de Vringer [29]. Glass beakers with a diameter of 3 cm were filled with 25 ml of ultrapure water and covered with filter paper (Whatman no: 42, cut off size: 2.5 µm, USA). After all beakers were sealed carefully with teflon tape and parafilm, 500 mg of each sample were spread regularly to obtain a thin film layer on the filter surface. The samples were kept at

32 °C for 24h in an incubator (Inkubator 1000, Heidolph, Germany) with constant relative humidity. Beakers coated with a filter paper but without an applied sample were served as control. All samples were weighed after 6th, 12th and 24th hours in order to give the water loss due to evaporation through the filter and the occlusion factor "F" was calculated according to the following equation (Equation 1). Where A is the water loss of without sample (control) and B is the water loss with the semi-solid lipid nanoparticle formulations. The F value of 100 means the maximum occlusiveness, while 0 means no occlusive effect compared to the control group. Each experiment was carried out in triplicate.

$$F = \frac{(A-B)}{A} \times 100 \qquad \text{(Eq.1)}$$

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