Journal of Pharmaceutical Investigation
Vol. 40, No. 6, 373-378 (2010)

Enhanced Occlusiveness of Nanostructured Lipid Carrier (NLC)-based Carbogel as a Skin Moisturizing Vehicle

Woo Sik Choi, Hye In Cho, Hyun Young Lee, Seo Hyun Lee and Young Wook Choi†

Division of Pharmaceutical Science, College of Pharmacy, Chung-Ang University, 221 Heuksuk-dong, Dongjak-gu, Seoul 156-756, Korea

(Received November 12, 2010 · Revised November 26, 2010 · Accepted November 27, 2010)

ABSTRACT − In order to develop a topical preparation which has a high occlusive property with skin moisturization, nanostructured lipid carrier (NLC) systems along with solid lipid nanoparticle (SLN) were designed. Various NLC dispersions were successfully formulated with Compritol 888 ATO as a solid lipid, Labrafil M 1944 CS as an oil, and Tween 80 as a surfactant. The increase of oil content (5 to 50%) led to the decrease in the occlusion factor in the order of SLN > NLC-5 > NLC-15 = NLC-30 > NLC-50. Particle size of lipid particulates was in the range of 100 to 160 nm. NLC-based carbogels were prepared by the employment of humectants such as urea, glycerin, and Tinocare GL to carbomer gel. NLC-30 gel formulations containing 4 or 8 % of lipid particles showed improved occlusive effect in vitro, compared to NLC-free gel base. Even though NLC-free gel base revealed comparable occlusion effect by itself, the occlusion factor of 4 % NLC-30 gel was about 2-fold higher than that of NLC-free gel base.

Key words − Carbogel, Lipid carrier, Nanoparticle, Moisturizer, Occlusion

Lipid-based carrier systems including nanostructured lipid carriers (NLC) and solid lipid nanoparticles (SLN) were introduced as an alternative for the existing traditional carriers such as emulsions, liposomes and polymeric nanoparticles (Müller et al., 1996). They have been widely studied for several routes of administration via peroral, parenteral, dermal and topical delivery (Wissing et al., 2003; Müller et al., 2004; Cavalli et al., 2003; Wissing et al., 2004; Souto et al., 2006). These carrier systems are non-toxic, since the NLC and SLN are prepared using biodegradable and biocompatible lipids. They can also load sufficient amounts of lipophilic drug. They have advantages of avoiding the use of organic solvents, feasibility of controlled drug release, drug targeting, and the ease of large scale production (Müller et al., 2000; Müller et al., 2002a; Mehnert et al., 2001). Especially in the topical administration, by virtue of smaller size of lipid particles, they can closely contact to stratum corneum and can increase the amount of drug penetrating into the skin. It is reported that the penetration of drug into human skin depends strongly on skin hydration which can be influenced by occlusive compounds (Ziegenmeyer et al., 1992) SLN or NLC forms a film after topical application, which in turn gives the occlusive effect on the skin surface to prevent water evaporation.

†Corresponding Author :
Tel : +82-2-820-5609, E-mail : ywchoi@cau.ac.kr
DOI : 10.4333/KPS.2010.40.6.373

SLN dispersion consists of solid lipid, surfactant, and water. Solid lipids including triglycerides, hard fats, fatty acid, and waxes are used to form homogeneous lipid matrix (Fig. 1). The solid lipid matrix of SLN can reduce the mobility of loaded drug molecules, and improves the stability of drug by protecting the sensitive groups of drug molecules (Kristl et al., 2003). Hence, SLN is a suitable carrier system for protecting drug from susceptible conditions such as oxidation or reduction. However, some limitations in SLN application are reported, due to limited drug loading by the low solubility of the drug in the solid lipid and drug leakage during storage caused by lipid polymorphism (Müller et al., 2002b). On the other hand, NLC dispersion consists of lipid blends, surfactant and water. Lipid blends are mixture of solid lipid and liquid oil. As shown in Fig. 1, in comparison to SLN, they generally form heterogeneous lipid matrix structure. Since the different structure of lipid blends and different solubility of drugs between oil and solid lipid, NLC shows higher drug loading capacity by virtue of greater solubility of drug in oil component.

For easy application of NLC dispersion onto the skin, semi-solid dosage forms including ointment, cream, or hydrogel could be employed. Carbomer (carbopol 934P), an acrylic acid polymer cross-linked with polyalkenyl polyethers or diviny glycol (Ding et al., 1998), is one of the commonly used hydrophilic polymers producing gel formulations. Carbomer is a very useful component for pharmaceutical gel systems, includ-

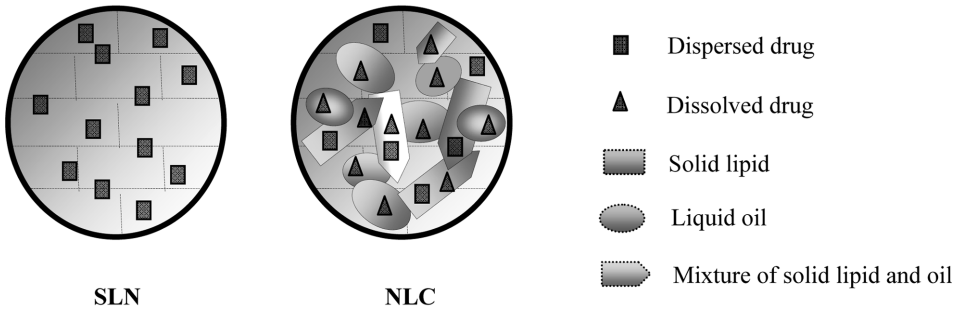


Figure 1. Drawings for homogeneous lipid matrix in SLN and heterogeneous lipid matrix in NLC.

ing buccal, transdermal, ocular, rectal, and nasal applications (Dittgen et al., 1997; Kumar et al., 1994; Morimoto et al., 1980; Chu et al., 1991). Especially, topical application of carbomer gels (carbogels) is favorable as they possess good rheological properties, resulting in long residual time at the site of administration. They offer good alternatives to oil-based ointment formulations. Carbogels are anionic hydrogels with good buffering capacity, which may contribute to the maintenance of the desired pH in the process of iontophoresis (Merclin et al., 2004). Moreover, it has some advantages such as high viscosity at low concentration, compatibility with many active ingredients, bioadhesive properties, good thermal stability, and good patient acceptance (Tamburic et al., 1995a, 1995b). Carbomer can resist physical stress caused by body or skin movement due to its gel structure being networked (Yonese et al., 2001).

The aim of this study was to develop an NLC-based carbogel system for enhancing occlusive effect by small lipid particles. Compositions of NLC dispersions are optimized with solid lipid and liquid oil and examined for the potential usefulness of NLC-based carbogel in terms of *in vitro* occlusion effect.

Materials and Methods

Materials

Compritol 888 ATO (glyceryl behenate) and Labrafil M 1944 CS (oleolyl macrogolglycerides) were kindly provided from Gattefosse (Korea). Poloxamer 188 and Tinocare GL were provided by BASF (Korea). Carbopol 934P was provide from Masung, Co., Ltd (Korea). Tween 80 and urea were purchased from Daejung Chemicals (Korea). Carnosine was purchased from Tokyo Kasei Kogyo Co., Ltd (Japan). All other chemicals and reagents were purchased from commercial sources and were of analytical grade. Double distilled water was used for all experiments.

Preparation of NLC dispersions

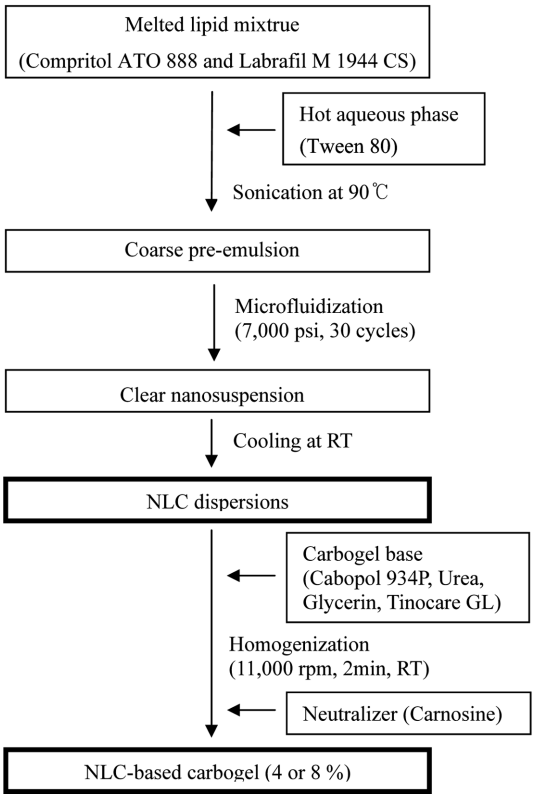
NLC dispersions were prepared by high pressure homogenization (HPH) method (Müller et al., 1996). Briefly, Compritol 888 ATO (solid lipid) and Labrafil M 1944 CS (oil) (total lipid 5 w/v %, varying the oil ratio) were melted together in a water bath maintained over 90ºC. Aqueous phase containing Tween 80 (2.5 v/v %) was also heated in the same water bath. Then the aqueous phase was added to the melted lipid mixture and the mixture was sonicated with a bath type sonicator maintained over 90ºC to produce coarse pre-emulsion with a texture of milky suspension. The obtained coarse pre-emulsion was then passed through a microfluidizer (7,000 psi, 30 cycles, Microfluidizer® M-110S, Microfluidics, Newton, MA, USA), resulting in clear nanosuspension. The nanosuspension was cooled at ambient temperature. The final formulations were stored in a refrigerator (Scheme 1).

Preparation of NLC-based carbogel formulations

The NLC-based gel formulations, containing 4 or 8% lipid particles were prepared (Scheme 1). As a gelling agent, Carbopol 934P (0.5%) was dispersed in the NLC dispersions containing 4 or 8 % lipid particles using a stirrer at a speed of 300 rpm for 5 min. Then urea (3%), glycerin (5%), and Tinocare GL (10%) were added to the dispersions as a humectant. The aqueous dispersions were homogenized using homogenizer at 11,000 rpm for 2 min. Finally, the aqueous dispersions were neutralized by carnosine (0.5%) to adjust pH of gel formulation in the range of 5.5 to 6.5.

Particle size and zeta potential of topical formulations

Both NLC dispersions and NLC-based gel formulations were diluted with an appropriate volume of water and were examined for particle size and zeta potential using dynamic light scattering particles size analyzer (Zetasizer Nano-ZS, Malvern Instrument, Worcestershire, UK) with 50 mV laser at a scattering angle of 90º. All measurements were carried out under ambient conditions and triplicated.



Scheme 1. Preparation of Nanostructured Lipid Carriers (NLC) and NLC-based carbogel.

In vitro occlusion test

In order to evaluate the occlusive effect of topical formulations, *in vitro* occlusion tests were performed (De Vringer et al., 1992). First, in case of NLC dispersions, beakers (100 mL) were filled with 50 mL of water, and covered with filter paper (cellulose filter, 42.5 mm, Whatman number 6, cutoff size : 3 μm, USA). Simultaneously, beakers were sealed with paraffin film for uncovered region. 300 μL of sample (NLC dispersion) was spread homogeneously with a spatula on the filter surface (9.63 cm^2). Then, in order to mimic the temperature of the skin surface, beakers were stored at 32°C and 50-55% relative humidity for 24 hr. The weight of water remaining in the beakers was measured at 6, 12, and 24 hr. Beakers in which 300 μL water was spread and treated under the same procedure were used as a reference. In case of NLC-based gel formulations, experiment was performed with the same method and instrument of NLC dispersions, except the amount of sample as 5 μL, in consideration of spreading ability of gel formulations. All experiments were performed in triplicates (n=3). The occlusion factor (F) was calculated by $F = [(A - B)/A] \times 100$, where A is the water loss in reference and B is the water loss in sample. An occlusion factor of zero means no occlusive effect compared to the reference, while occlusion factor of 100 means maximum occlusive effect.

Statistical analysis

All reported data are mean ± S.D. Statistical significance was checked by Student's t-test and considered to be granted at $P<0.05$, unless otherwise indicated.

Results and Discussion

Composition of NLC dispersions

Various NLC dispersions were formulated with solid lipid, oil, and surfactant (Table I). The total amount of lipid phase (Compritol 888 ATO as a lipid and Labrafil M 1944 CS as an oil) was kept constant at 5%, while the percentage of oil content was varied from 0 to 50% versus the amount of total lipid. All components should be appropriate for skin administration. First of all, Compritol 888 ATO (glyceryl behenate) was selected as a solid lipid. The structure of glyceride of behenic acid is suitable for topical application, since the lipid matrix of the stratum corneum is composed of ceramides, free fatty acids and cholesterol (Schurer et al., 1991). It is also previously reported that Compritol 888 ATO has higher occlusive properties than other solid lipids (Dynasan 112 and Softisan 154), since it has higher crystallinity than other lipids (Wissing et al., 2002). Labrafil M 1944 CS is the glyceride ester of apricot kernel oil, which is rich in fatty acids of oleic or linoleic acid and Vitamin A. It is suitable for either sensitive or dry skin, because of both emollient and nourishing effects. It is also beneficial in relieving the itchiness caused by eczema. Finally, Tween 80 was selected as a surfactant in consideration of low skin irritation of nonionic surfactant.

Physical characteristics of NLC dispersions

The physical characteristics of NLC dispersions were evaluated by particle size, polydispersity index, and zeta potential (Table II). Among these properties, particle size is an important

Table I. *Composition of the Developed NLC Dispersions (%)*

	SLN[a)]	NLC[b)]-5	NLC-15	NLC-30	NLC-50
Compritol ATO 888	5	4.75	4.25	3.5	2.5
Labrafil M 1944 CS	-	0.25	0.75	1.5	2.5
Tween 80	2.5	2.5	2.5	2.5	2.5
Water q.s. ad	100	100	100	100	100

[a)]SLN represents for oil-free composition, [b)]NLC formulations were designated as the percentage of oil content versus the amount of total lipid.

Table II. *Physicochemical Characteristics of NLC Dispersions.*

	SLN	NLC-5	NLC-15	NLC-30	NLC-50
Size (nm)	143 ± 8	156 ± 6	152 ± 3	132 ± 1	109 ± 4
PDI*	0.36	0.42	0.44	0.5	0.48
Zeta potential (mV)	-20.9 ± 1.3	-24.3 ± 0.7	-17.9 ± 0.3	-16.9 ± 0.8	-22.4 ± 0.5

*polydispersity index. Data represent mean ± SD (n=3).

factor, since occlusive property was affected by particle size. As shown in Table II, the particle size was measured as approximately 100 to 160 nm in average, which is considered as an ideal size for excellent occlusive effect. When occlusive effect was evaluated by varying particle size from 200 to 4000 nm, particles of 200 nm size range show the highest occlusive effect (Wissing et al., 2003). Regardless of oil content, the size of NLC particles was in similar range. Surface charge of NLC dispersions was measured as a slight negative value. Hence, all NLC dispersions were suitable for further study.

In vitro occlusive effect of NLC dispersions

Since small particles possess an adhesive effect, the adhesion increases with decreasing particle size. This principle applies for all types of particulate carriers, including liposomes, NLC, and SLN, by the formation of film after topical application. Intensive *in vitro* studies were performed to quantify the occlusivity of NLC and SLN in the form of the so-called 'occlusion factor' (De Vringer et al., 1992). When NLC or SLN dispersion is applied on the filter surface, a thin film is formed to prevent water evaporation through the filter paper, rendering occlusive properties (Müller et al., 2002). While SLN forms a perfect and homogeneous lipid matrix, NLC produces an imperfect and heterogeneous lipid matrix. Therefore, in general, SLN has higher occlusive effect than NLC. The *in vitro* occlusion factors of various NLC dispersions are shown in Fig. 2. The order of occlusion factor was revealed as follows: SLN > NLC-5 > NLC-15 = NLC-30 > NLC-50. In case of NLC dispersions, the increase of oil content led to the decrease in the occlusion factor due to the higher degree of imperfection of lipid matrix. However, NLC-30 was selected for the encapsulations of lipophilic drug. Although NLC has lower occlusive effect compared to SLN, it has an advantage of high drug loading due to greater solubility of drug in oil.

Properties of NLC-based carbogel

NLC-based carbogel was developed for topical application of NLC, because of the difficulty for applying NLC dispersion onto the skin. In order to form a polymeric hydrogel network, carbomer was expanded in aqueous medium by neutralization. It is possible to neutralize polymers by employing organic

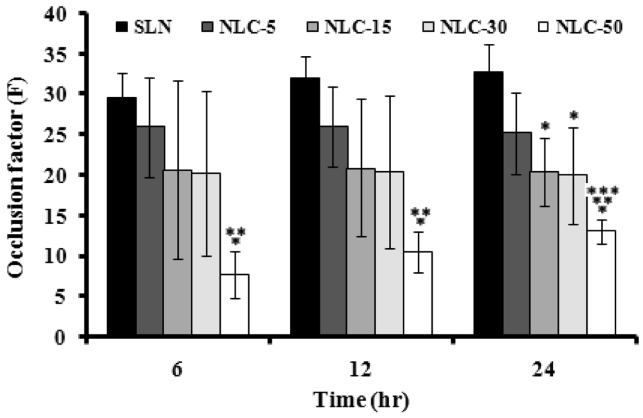


Figure 2. Occlusion factors of SLN and NLC dispersions. Data represent mean ± SD (n=3) and the statistical analysis was performed using the Student's t-test (*P<0.05 versus SLN; **P<0.05 versus NLC-5; ***P<0.05 versus NLC-15).

amines as a neutralizing agent. In general, triethanolamine and sodium hydroxide are the examples. However, because of allergic contact dermatitis and erosion for the above neutralizing agents (Scheuer et al., 1983), carnosine is used as an alternative in this study. Carnosine is a basic dipeptide of the amino acids beta-alanyl-L-histidine, which has a beneficial antioxidant property. Additional excipients including urea, glycerin, and Tinocare GL are used as a humectant, which is absorbed into the stratum corneum and increases skin hydration by the attraction of water in the air (Flynn et al., 2001). For NLC-based gel, the particle size and polydispersity index were measured as approximately 240 nm and 0.6, respectively. Compared to NLC dispersions, particle size and polydispersity index were slightly increased. It was interpreted as the same reason that carbomer induced a slight aggregation between lipid particles (Jenning et al., 2000). However, based on the report that lipid particles have similar occlusive effect up to 400 nm (Wissing et al., 2003), NLC-based carbogel was used for further study.

In vitro occlusive effect of NLC-based carbogel

The occlusion factors of various NLC-based carbogels are shown in Fig. 3. NLC-30 gel formulations containing 4% or 8% of lipid particles showed improved occlusive effect compared to gel base alone. The order of occlusion factor was

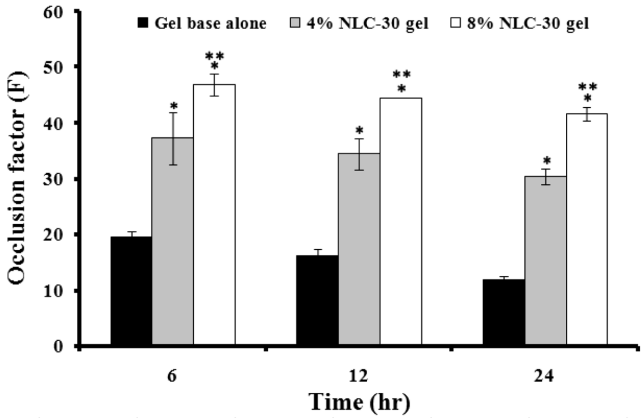


Figure 3. Occlusion factors of NLC-based gels and gel base alone. Data represent mean ± SD (n=3) and the statistical analysis was performed using the Student's t-test (*P<0.05 versus Gel base alone; **P<0.05 versus 4% NLC-30 gel).

revealed as follows: 8% NLC-30 gel > 4% NLC-30 gel > gel base alone. It is shown that the increase in loading of lipid particles led to the increase in the occlusion factor, because of the increased amount of lipid particles to cover filter surface. The occlusion factor of 4% NLC-30 gel was about 2-fold higher than that of gel base alone. However, we have to notice that NLC-free gel base also showed comparable occlusion effect by itself. On the other hand, 8% NLC-30 gel revealed increased viscosity, causing uncomfortable spreadability on skin application. Therefore, it is considered that improved occlusive effect was achieved with 4% NLC-30 gel formulation.

Conclusion

This study indicates that various NLC dispersions were successfully formulated with Compritol 888 ATO as a solid lipid, Labrafil M 1944 CS as an oil, and Tween 80 as a surfactant. The increase of oil content led to the decrease in the occlusion factor due to higher degree of imperfection of lipid matrix. Considering solubility of lipophilic drug in oil, NLC-30 dispersion was selected for drug loading and carbogel formulation for topical application. NLC-based carbogels were formulated by the employment of humectants such as urea, glycerin, and Tinocare GL to carbomer gel. Pharmaceutical properties were greatly improved in terms of *in vitro* occlusion.

Acknowledgements

This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A091121).

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