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# Effects of Oil type on the Stability of Oil-in-Water Lipid Nanoemulsion

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**Abstract** : Nanoemulsions are actively used in several applications for pharmaceutical, cosmetic and chemical industries. In this study, we propose the use of microfluidizer known as high pressure homogenizer to prepare lipid nanoemulsion as a potent cosmetic delivery carrier. The lipid nanoemulsions were prepared by O/W emulsion with hydrogenated lecithin and different type of oils. Effects of oil type on the stability of the lipid nanoemulsion were investigated with Dynamic Light Scattering (DLS) and Zeta-potential. Arbutin was used as model drug for transdermal administration through hairless mouse skin. Transdermal arbutin delivery using the lipid nanoemulsions was studied with HPLC method.

*Keywords : Lipid Nanoemulsion, Microfluidizer, Transdermal delivery, Arbutin*

## 1. Introduction

Generally, emulsions have been classified as macro, micro, and nano-emulsion, according to their droplet sizes of 0.1 ~100 $\mu$m. Among various emulsions system, nanoemulsions are in the spotlight of application in cosmetic and pharmaceutical industries [1-3]. Nanoemulsions are thermodynamically stable, and they show high solubilizing capacity, so that it facilitates the skin permeation of both hydrophilic and hydrophobic drugs [4]. Many factors such as components, compositions, preparation method and formulation conditions, are known to change the stability of emulsions. It has been a long-standing aim to formulate stable emulsions with small particles since the stabilization of emulsion could be achieved by particle size reduction [5-7]. Nanoemulsions as colloidal drug carriers are interesting in the field of drug delivery systems because their small size allow them to permeate through biological barriers [2, 8-11]. Also, it has been playing an important role to protect drugs against degradation. Among various colloidal drug delivery systems, lipid nanoemulsions potentially represent one of the most promising approaches to this aim [3, 9, 12-13]. It is one of the most important problems to avoid their denaturation during the transport to their site action [14]. Lipid nanoemulsions generally have prepared by emulsification machine such as homomixer, homogenizer and microfluidizer [15]. In order to prepare lipid nanoemulsions with lower size polydispersity, we used

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microfluidizer known as high pressure homogenizer. The microfluidization process involves flow of the liquid mixture at very high pressure through micro channels toward an impingement area. Precisely controlled emulsification forces generated by this technique include high shear (laminar flow), turbulence (inertial flow), and cavitation (vapor bubble implosion) with these mechanical forces acting together to reduce mean droplet diameter of the dispersed phase [16-17]. The purpose of this study was to formulate lipid nanoemulsions and study the influence of the processing technique such as homogenization and microfluidization on the initial droplet diameter and rate of droplet aggregation in an oil-in-water emulsion system with respect to various oil types. Also, we investigated the effect of various oil types on the skin permeability.

## 2. Experimental

### 2.1. Materials

The hydrogenated lecithin emulsifier used was Lipoid S75 kindly donated by Altwell Cosmetic Co. (Seoul, Korea). Arbutin (Sigma Chemical Co.) was used as model drug for transdermal administration through hair-less mouse skin. The different oil types used were kindly provided by Altwell Cosmetic Co. (Seoul, Korea). The name of oil types used were Mineral oil (D-7), Triethylhexanoin (TIO), Cetyl ethylhexanoate (CIO), Triglyceride (MCT), Squalane (SQ), Dimethicone (6CS), Phenyltrimethicone (DC-556), Octyldodecanol (Octyldo), Octyldodecyl myristate (ODM), Isocetyl ethylhexanoate (ICEM), and Isocetyl myristate (ICMR). In order to prepare PBS solution, phosphate buffered saline, pH 7.4 was purchased from Sigma Chemical Co. Distilled water was of Milli-Q quality (Millipore, USA-Bedford, MD). All organic solvents were either HPLC grade or American Chemical Society analytical grade reagents.

### 2.1. Preparation of Lipid Nanoemulsion

Lipid nanoemulsions with arbutin were prepared by microfluidizer known as high pressure homogenizer. A schematic presentation of the preparation process is shown in Fig. 1. In brief, 1000 mg of hydrogenated lecithin was dissolved in 100 ml of D.D.I. water, and then 500 mg of arbutin was added. The mixture was mixed for 5 min with agitator. 10 g of oil was added into the mixture. After mutual saturation of organic and continuous phases, the mixture was emulsified for 5 min with a homo mixer (about 3,000 rpm - 4,000 rpm), and then water is to o/w emulsion solution. This was added into the microfluidizer known as high pressure homogenizer at 1500 bar.

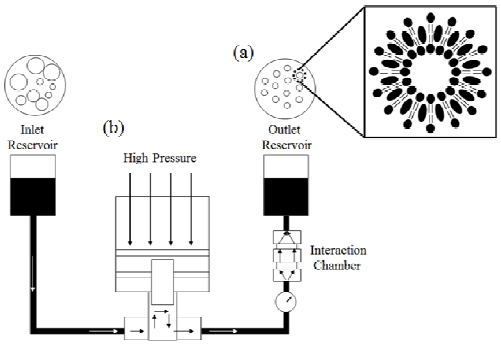


Fig. 1. Schematic representation of the (a) lipid nanoemulsion preparation using (b) a microfluidizer.

### 2.2. Characterization of Lipid Nanoemulsion

#### 2.2.1. Particle size analysis and zeta potential

Zeta potentials of nanoemlusion adsorbed with lipid were measured by ZetaPlus (Brookhaven Instruments Co., Holtsville, New York) with Uzgiris, Brookhaven electrodes coated by palladium, and He-Ne laser as a light source. Each data point for zeta potentials is an average of at least 15 measurements. Mobility of nanoemulsion is calculated from a shift in frequency of a laser Doppler spectrum. Consequently, zeta potential

(z) was calculated by the simple Smoluchowski equation

$$\mu = \frac{\varepsilon_0 \varepsilon_w \zeta}{\eta} \quad [1]$$

where $m$ is the mobility ($10^{-8}$ ·$m^2$· $s^{-1}$ ·$V^{-1}$), $e_0$ is the permittivity of free space ($C^2 \cdot J^{-1} \cdot m^{-1}$), $e_w$ is the relative permittivity of water, $h$ is the viscosity of water ($g \cdot cm^{-1} \cdot s^{-1}$) and $z$ is the zeta potential (mV). The half width at half height of calculated zeta potential was less than 15 mV in all the cases. The zeta potential of lipid nanoemulsion of the different oil types were measured. For analysis of particle size on the time of lipid nanoemulsions which were prepared by microfluidizer, 0.5 ml of o/w lipid nanoemulsion was diluted to 5 ml with D.D.I. water. The particle size of nanoemulsion was measured by dynamic light scattering method based on the particle size option in ZetaPlus. The scattered intensity was registered at the scattering angle of 90° and temperature of 298.15K. All measurements were done at room temperatures.

#### 2.2.2. Interfacial Tension

The interfacial tensions were measured with a K8tensionmeter (Krüss) equipped with a du Nouy ring. All the experiments were repeated at least three times to all samples, and an average of all the values was determined. All measurements were carried out at 25 °C.

#### 2.2.3. Procedure for skin permeation in vitro

Male hairless mice aged 5-7 weeks were used. After sacrificing, full thickness of the dorsal skin of each hairless mice was carefully excised and subcutaneous fat was removed with a dull scalpel. The full skin thickness was measured using dial thickness gauge (Mitutoyo, Japan, 0.01-10mm). Appropriate sized pieces of skin were then mounted on Franz diffusion cells with a surface area of 2.2 $cm^2$ and a receiver capacity of 5 ml (labfine Co., Korea). The epidermal side of the skin was exposed to ambient conditions while the dermal side was bathed by isotonic phosphate buffer, pH 7.4. Care was exercised to remove any air bubbles between the underside of the skin and the receiver solution. The temperature of the receiver solution was maintained at 37 ℃. At predetermined times intervals, 300 μl lipid nanoemulsion of receiver solution was spread on the skin mounted on Franz diffusion cells and were stirred continuously by a small Teflon-coated magnetic bar to keep them well mixed, and the amount of arbutin was analyzed by HPLC (Waters 2690 Separations Module / Water 2487 A.D.). All experiments were carried out under non-occluded conditions.

## 3. Results and Discussion

### 3.1. The effect of different oil types on the dispersion stability

As the different oil types, the dispersion stability of lipid nanoemulsions which were prepared by microfluidizer was investigated and used the hydrogenated lecithin as the emulsifier and stabilizer. DLS was used to investigate suspension stability of the time at the high temperature, room temperature and low temperature (Fig. 2). Generally, all the oil types divide into siloxanes oil, liquid paraffin oil, alcohols oil, and esters oil. In this study, 556 and 6CS are siloxanes oil types. D-7 and SQ are liquid paraffin oil types. Alcohols oil type is octyldo. CIO, MCT, ICEM, ICMR, TIO, and ODM are esters oil types. In the chemical class, siloxanes oil has siloxane polymers in a structural formula. Liquid paraffin oil is also hydrocarbons obtained from petroleum or the saturated branched chain hydrocarbon obtained by hydrogenation of natural oils that conforms to the formula. Esters oil type has also ester in a structural formula (Table 1, The Cosmetic Toiletry Fragrance Association, 2002).

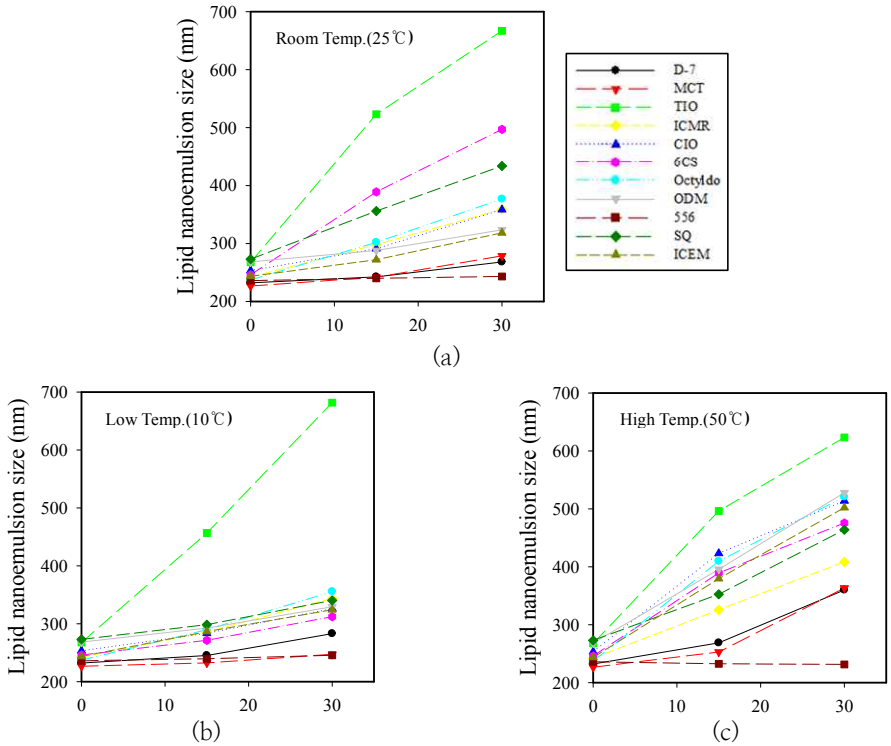


Fig. 2. Results of suspension stability with DLS as the time at the (a) 25℃, (b) 10℃ and (c) 50℃.

According to suspension stability of the time at the high temperature, room temperature and low temperature (Fig. 2), 556 of all others are the best stable oil at all temperature condition. As we know, the hydrogenated lecithin which is used in this process plays an important part in vesicle formation. Interface tension is also important factor in formation of lipid emulsion. But, according to analysis results, because microfluidizer which is called high pressure homogenizer has a mechanical property to make a very small lipid emulsion, Interface tension doesn't play an important role in formation of lipid emulsion. Anyway, according to analysis results of Interface tension (Fig. 3 and Table 2), esters oil type form smaller than other types in an initial formation of lipid emulsion.

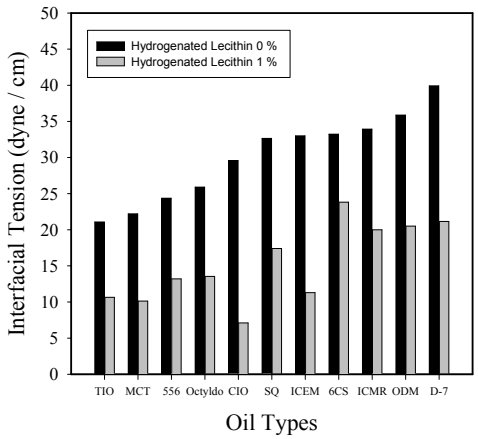


Fig. 3. Interface tension analysis of the hydrogenated lecithin concentration on the different oil types.

Table 1. Chemical property of the different oil types

| Oil abbreviation | Oil name | Chemical class | Definition |
|---|---|---|---|
| 6CS | Dimethicone | Siloxanes and Silanes | Dimethicone is a mixture of fully methlated linear siloxane polymers and blocked with trimethylsiloxy units. It conforms generally to the formula |
| 556 | Phenyl Trimethicone | Siloxanes and Silanes | Phenyl Trimethicone is the siloxane polymer that conform generally to the formula. |
| SQ | Squalane | Hydrocarbons | Squalane is the saturated branched chain hydrocarbon, obtained by hydrogenation of shark liver oil or other natural oils. |
| D-7 | Mineral oil | Hydrocarbons | Mineral oil is a liquid mixture of hydrocarbons obtained from petroleum. |
| CIO | Cetyl Ethylhexanoate | Esters | Cetyl Ethylhexanoate is the ester of cetyl alcohol and 2-ethylhexnoic acid. |
| ICEM | Isocetyl Ethylhexanoate | Esters | Isocetyl Ethylhexanoate is the ester of isocetyl alcohol and 2-ethylhexanoic acid. |
| ICMR | Isocetyl Myristate | Esters | Isocetyl Myristate is the ester of isocetyl alcohol and myristic acid. |
| ODM | Octyldodecyl Myristate | Esters | Octyldodecyl Myristate is the ester of Octyldodecanol and myristic acid. |
| TIO | Triethylhexanoin | Esters | Triethylhexanoin is the triester of glycerin and 2-ethylhexanoic acid. |
| MCT | Caprylic/Capric Triglyceride | Esters | Caprylic/Capric Triglyceride is the mixed triester of glycerin and caprylic |
| Octyldo | Octyldodecanol | alcohol | Octyldodecanol is an aliphatic alcohol. |

Zeta potential was used to investigate dispersion stability of the different oil types (Fig 4). Generally, an initial formation and long term stability are important factors in the dispersion stability. According to analysis results of DLS, interface tension, and zeta potential, we could suppose an initial formation and long term stability of lipid emulsion. Therefore, according to analysis results of DLS, interface tension, and zeta potential, esters oil type form smaller than other types in an initial formation of lipid emulsion. Siloxanes oil type is also more stable than other types in long term stability of lipid emulsion.

### 3.2. In vitro studies of transdermal administration

The nanoemulsions used in these studies were prepared with an aqueous phase consisting of arbutin in distilled water and a hydrogenated lecithin. A various oils and a

Table 2. Interfacial tension analysis of the hydrogenated lecithin concentration on the different oil types

| Oil types | hydrogenated lecithin 0 % (dyne / cm) | hydrogenated lecithin 1 % (dyne / cm) |
|---|---|---|
| TIO | 21.0750 | 10.6500 |
| MCT | 22.2000 | 10.1250 |
| 556 | 24.3667 | 13.2000 |
| Octyldo | 25.9000 | 13.5500 |
| CIO | 29.5750 | 7.1000 |
| SQ | 32.6500 | 17.4000 |
| ICEM | 33.0000 | 11.3000 |
| 6CS | 33.2252 | 23.8250 |
| ICMR | 33.9250 | 20.0000 |
| ODM | 35.8750 | 20.5000 |
| D-7 | 39.9250 | 21.1500 |

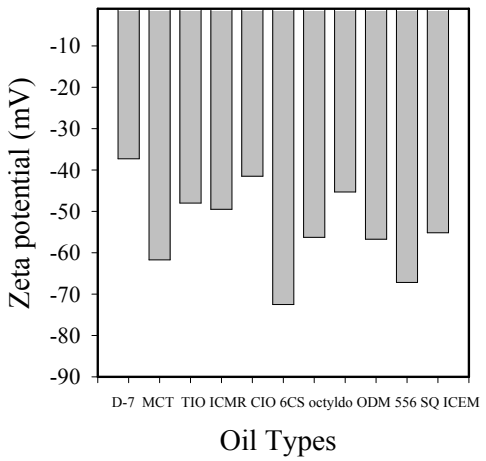


Fig. 4. Zeta-potential of dispersion stability as the different oil types.

small lipid emulsion size have known to be enhancers the skin permeability. We investigated the effect of various oils on the skin permeability and the effect of microfluidizer and homogenizer. Eleven oil types consist of D-7, TIO, CIO, MCT, SQ, 6CS, 556, Octyldo, ODM, ICEM and ICMR. Percutaneous absorption was monitored for total period of 2 - 24 h (Fig. 5). As shown in Figure 5, although the dispersion stability of oil was excellent, the arbutin percutaneous absorption content was not always excellent. It can be explained that a hydrophobic and a hydrophilic property with oil different. Generally, all the oil types divide into siloxanes oil, liquid paraffin oil, alcohols oil, and esters oil and as the chemical structure with each other oil, these oil types have a different hydrophobic property and a different hydrophilic property. Therefore, as a hydrophobic and a hydrophilic property of oil have influence on percutaneous absorption content of arbutin. As shown in Figure 5, although the hydrophobic property of oil at chemical structure was excellent, the arbutin percutaneous absorption content was not always excellent. Also, although the hydrophilic property of oil at chemical structure was excellent, the arbutin percutaneous absorption content was not always excellent. Therefore, in order to increase percutaneous absorption content of arbutin, a hydrophobic oil and a hydrophilic oil should be mixed optimum condition each other. According to the arbutin percutaneous absorption contents of the oil types as the formulation methods, microfluidizer was

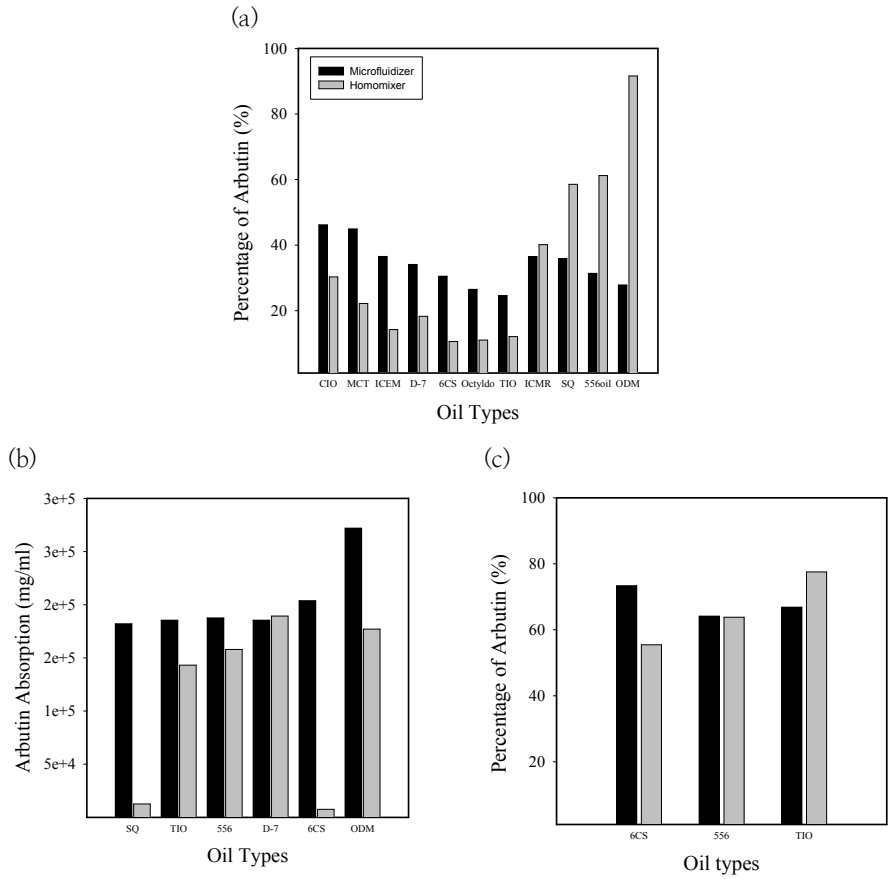


Fig. 5. The effect of percutaneous absorption content of arbutin with hairless mice skin as the oil types and processing methods. (a) 2hr, (b) 12hr, (c) 24hr.

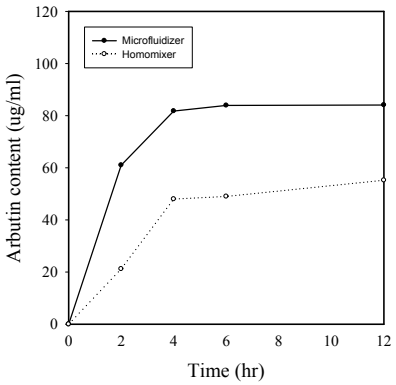


Fig. 6. The result of figure 6 especially means he arbutin percutaneous absorption contents of 6CS oil type of microfluidizer and homogenazier on the time (2, 4, 6, and 12hr).

comparatively better than homogenizer in the percutaneous absorption of arbutin (Fig. 5 and Fig. 6). According to percutaneous absorption test of time (2, 4, 6, and 12 hr), burst effect appeared to be observed in diffusion phenomenon of drug. Although lipid nanoemulsion appeared burst effect at the first, keeping stability of drug was comparatively excellent during 12 hr.

## 4. Conclusions

Lipid nanoemulsions with arbutin were prepared by microfluidizer and homogenizer as the different oil types. Microfluidizer which is

called high pressure homogenazier made more stable and smaller lipid emulsion shape than homogenizer.

According to analysis results of DLS, interface tension, and zeta potential, esters oil type form smaller than other types in an initial formation of lipid emulsion. Siloxanes oil type is also more stable than other types in long term stability of lipid emulsion.

According to percutaneous absorption test, burst effect appeared to be observed in diffusion phenomenon of drug.

Microfluidizer can make more stable and smaller than homogenizer in the formation of lipid emulsion and was better than homogenizer in the percutaneous absorption of arbutin.

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