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# Nanosolve와 PMMA를 이용한 유용성감초산의 안정화에 대한 연구

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# The Study for Stability of Useful *Glycyrrhiza uralensis* (Licorice Root) Using Nanosolve and PMMA

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요 약: 유용성 감초산은 강한 항염 작용으로 주름개선용 기능성 화장품에서 널리 사용되어지는 물질이다. 그러나 용해성이 좋지 않고 빛, 열, 산소에 의하여 변질되기 쉽다. 본연구에서는 유용성감초산을 PG/hydrogenated lecithin/MCT/glycerine/water를 microfluidzer를 이용해 30~50 nm인 nanosolve-licorice를 만들고, 잘 용해된 nanosolve-licorice를 다공성 PMMA에 에탄올과 함께 넣은 후 microfluidzer를 1000 BAR에서 연속 3회 처리하여 안정화시켰다. 이 실험에서 글라블리딘 함량을 HPLC로 측정한 결과 기존의 리포좀에 비하여 3~5배 정도 안정성을 보였으며, PMMA 캡슐로 된 리코리스는 피부의 침투가 기존 리포솜에 비하여 월등히 우수하여 항염 효과를 더욱 증가시켰다. 이러한 실험을 image analyzer, particle size analyzer, FF-TEM, chromameter, HPLC 등의 분석장비를 사용하였다

**Abstract:** *Glycyrrhiza uralensis* (licorice root) is very useful medicinal herb because of strong anti-inflammatory and anti-wrinkle effect. Therefore, it is widely used in functional cosmetics. However, it is insoluble and easily decomposed by light, heat, oxygen, etc. In this study, we first prepared NanoSolve-Licorice (30~50 nm) using *Glycyrrhiza uralensis* and propylene glycol/ hydrogenated lecithin/caprylic/capric triglyceride/glycerin/water system with microfluidizer. And then, NanoSolve-Licorice and porous PMMA are dispersed in ethanol. Finally, we could get a stabilized system with high-pressure homogenizer (1,000 Bar, 3 passes). According to HPLC measurement for glabridin content, our system is more stable compared with general liposome ones. Capsulated licorice has an enhanced anti-inflammatory effect on account of excellent skin penetration. We also evaluated our final product through image analyzer, particle size analyzer, FF-TEM and Chromameter.

***Keywords:*** *Glycyrrhiza uralensis (licorice root), liposome, nano-emulsion, microfluidizer, PMMA (polymethylmethacrylate)*

## 1. Introduction

The recent trend of cosmetics field is pursuing high functional ingredients and concept for well-being. Even though many active ingredients have a good efficacy and effect, they cannot be widely used because of their instability caused by environmental factors such as light, heat, oxygen, etc. Therefore, new technology and method are demanded to improve the stability of active materials. Based on this need, we have studied licorice root that have strong anti-inflammatory, whitening effects, anti-ulcerative activity and inhibitory effects on histamine release.

*Glycyrrhiza uralensis* fisch (Xibei licorice), *Glycyrrhiza glabra* Linne (Russian licorice), *Glycyrrhiza inflata* Bat. (Xinjarg licorice) is a perennial herb, belongs to

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leguminosae and has been used as a medical and table use. Its stem has white unicellular trichome, the shape of rhizome is cylindrical, main root is long and rough and its color is red to dark brown. A main elements of *Glycyrrhiza uralensis* extract are glycyrrhizine, glycyrrhetic acid, licoricidin and so on. They have the following effects: anti-inflammation, counteracting poison, anti-ulcer and hormone operation. Glycyrrhizine has been used as a sweetener.

In this study, licorice is stabilized by nanosolve and PMMA (polymethylmethacrylate). Liposome is spherical closed vesicles of phospholipid bilayers with entrapped water phase. Phospholipid composed of lipid membrane is amphipathic. It has two hydrophobic fatty acid groups and one hydrophilic phosphate group. Therefore phospholipid forms bilayer in water phase and can have spherical closed vesicles. In this bilayer, tail parts (non-polar fatty acid groups) are heading to inside of membrane and head parts (polar) are heading to outside of membrane. Liposome is divided into two classes based on the number of lamella: MLV (multi-lamellar vesicles) having more than five lamellas and 100~1,000 nm in diameter and ULV (uni-lamellar vesicles) having LUV (large uni-lamellar vesicles), over 100 nm and SUV (small uni-lamellar vesicles), below 100 nm. Liposome in cosmetics field is mainly organized with phospholipid that has excellent skin affinity to enhance the stability against light, heat, oxygen, etc. and the penetration into skin. A lot of researches on DDS (drug delivery system) through the liposome of licorice have been achieved but there is no full investigation about stability.

So we are focusing the licorice stability using nanoemulsion (primary liposome)[1-3] with hydrogenated lecithin (having excellent skin affinity). Primary liposome is again capsulated with PMMA. In this manner, obtained PMMA-liposome is investigated using cryo-SEM, TEM to identify the formation of liposomes and laser light scattering system to measure the liposomes size. Chromameter and HPLC are used to check the color and glabridin content change and against heat and light, respectively. Skin penetration effect is also measured to compare with general liposome system.

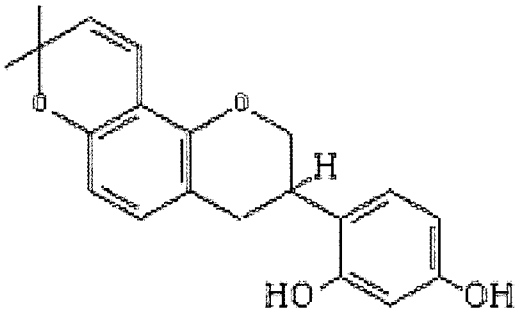


**Figure 1.** The structure of glabridin.

## 2. Materials and Methods

### 2.1. Equipment

We checked the color change of PMMA-liposome using chromameter (CM-1000 R, Minolta, Osaka, Japan) and measured the particle size and Zeta potential using laser light scattering system (Zetasizer 300 M, Malvern Ltd. UK). Microfluidizer (M 110 F, Microfluidics Corp. USA) and TK Auto Homomixer (Tokusbu, Kika, Kogyo, Japan) are employed for emulsification. Transmission electron microscope (JEM 1010, Jeol, Japan) and HPLC (Model 510, Waters, USA) are used to investigate particle and analyze glabridin content respectively. Steel (250×4.6 mm) as a column and hypersil-octadecylsilane silica gel (282 nm) as a stationary phase have been used.

### 2.2. Materials

For this experiment, Lipoid S 75-3 (hydrogenated lecithin) of Lipoid company and *Glycyrrhiza uralensis* (Maruzen, oil-soluble licorice EXT P-T (40); glabridin 40%; Figure. 1) are used. All other material used in our experiment is raw material for cosmetics. Finally, we used purified water that had passed through an anion-cation exchange resin column.

### 2.3. Method

We first prepared nanosolve-licorice (30~50 nm) using *Glycyrrhiza uralensis*/propylene glycol/hydrogenated lecithin/caprylic/capric triglyceride/glycerin/water with microfluidizer. And then, nanosolve-licorice and porous PMMA are dispersed in ethanol. Finally, we could get a stabilized system with high-pressure homogenizer (1,000 Bar, 3 passes).

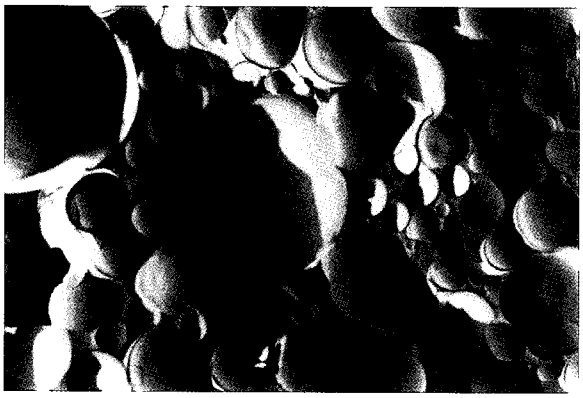

**Figure 2.** FF-SEM image of PMMA-liposome.

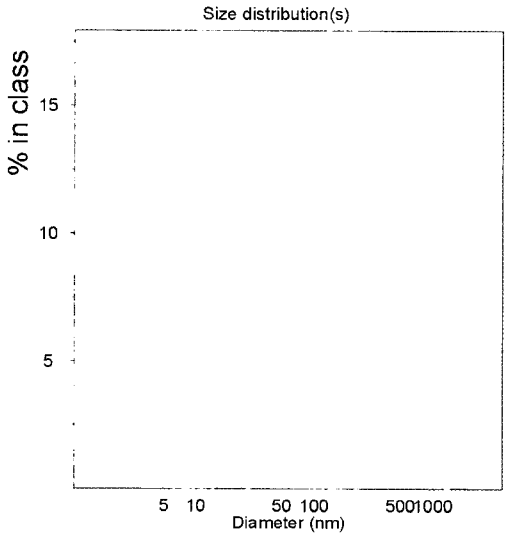


**Figure 3.** Particle size distribution of PMMA-liposome.

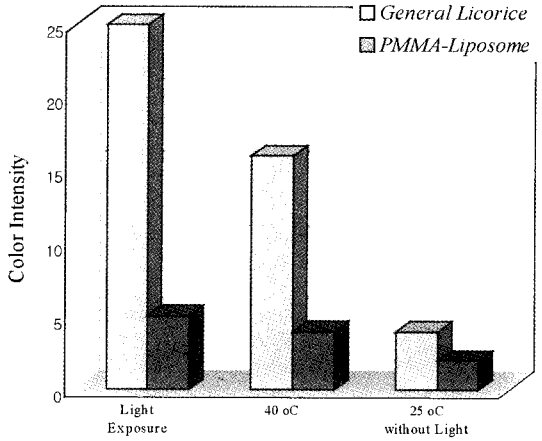


**Figure 4.** Color stability of licorice and PMMA-liposome against heat and light.

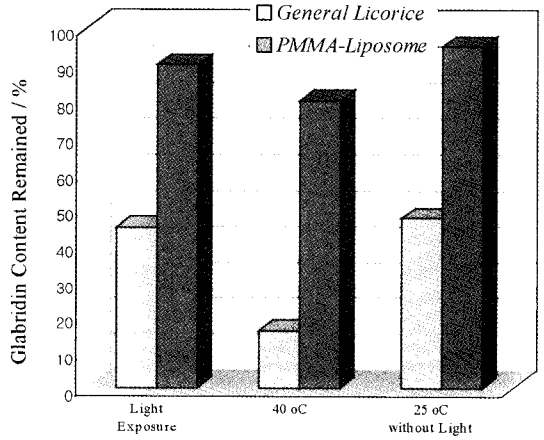


**Figure 5.** Variation of glabridin content.

## 3. Results and Discussion

### 3.1. Identification of Liposome Formation

TEM and FF-SEM (freeze-fracture scanning electron microscopy) are used to identify the formation of liposome. As shown in Figure 2, it is seen that nano-emulsion and liposome are well formed by NanoSolve. PMMA give them more stability.

### 3.2. Particle Size Distribution of PMMA-liposome

We also studied for particle size distribution of liposome with laser light scattering system, which is shown in Figure. 3. Liposome has the size distribution from 20 to 50 nm and mean diameter about 30 nm. Also we observed that NanoSolve capsulated with PMMA is formed from 30 to 1,000 nm and mean diameter about 300 nm.

### 3.3. Color Stability of Licorice and PMMA-liposome Against Heat and Light

Licorice and PMMA-liposome are stored for one month under following conditions: 25℃+without light, 40℃ and light exposure. In one month, color change is measured by chromameter. As shown in the Figure. 4, we observed that color of licorice and PMMA-liposome varied with heat and light as time goes by. Under light exposure condition, PMMA-liposome is more stable by about 5 times compared with licorice. Under 40℃ and 25℃+without light condition, it is 4 times and 2 times, respectively. These results mean that PMMA-liposome is more stable than licorice only because it is stabilized by means of porous PMMA that can give

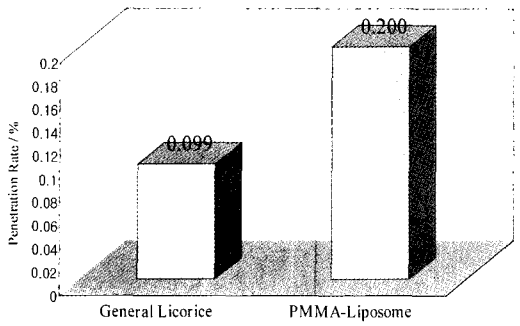


**Figure 6.** Skin penetration effect.

nano-emulsified licorice more stability.

### 3.4. Variation of Glabridin Content

Variation of glabridin content for two month is shown in Figure 5. It was showed that the stability of PMMA-liposome is enhanced by 50% compared with licorice only under light exposure. And improved stabilities are 80% at 40℃ and 50% at 25℃+without light. The reason for improved stability is the same of color stability.

### 3.5. Skin Penetration Effect of Licorice and PMMA-liposome

An 8-week-old hairless guienia pig (strain IAF/HA-hrbp) is used to study skin penetration effect. We put the abdominal skin of guienia pig cut off in Franz-type diffusion cells (Lab Fine Instruments). Receptor of cell (5 mL) is filled with 50 mM phosphate buffer (pH 7.4 and 0.1 M NaCl). Solution is mixed and dispersed with 600 rpm while the cell temperature is keeping at 32℃. Produced solution (200 μm) is poured in donor container. As prearrangement timetable, solution is absorbed and diffused. At this time, skin area absorbed and diffused is 0.64 $cm^2$. After absorption and diffusion of active ingredient, the remainder on skin is washed off with wetted kim-wipes using 10 mL purified water. And then skin that active ingredient is absorbed and diffused is grinded with tip-type homogenizer (Polytron PT 2100, Switzerland). Licorice penetrated into skin is extracted using 4 mL ethanol. An extract (0.45 μm) is filtered by nylon filter membrane and Licorice content is measured by HPLC.

Two different types of cream containing 2 wt% of licorice and PMMA-liposome are prepared, respectively. After we applied the cream onto the skin of a hairless mouse, glabridin content is measured by HPLC. As shown in Figure 6, skin penetration of licorice is about 0.099% and that of PMMA-liposome is about 0.200%. Its penetration efficiency is enhanced by 2 times. In terms of this result, we could think PMMA-liposome improves skin penetration of glabridin because its structure materials are similar to skin structure ones.

## 4. Conclusion

In order to improve licorice stability, we introduced PMMA-liposome system which is prepared by combining NanoSolve and PMMA. Liposome is 20~50 nm in size (mean diameter ~30 nm). PMMA-liposome is 30~1,000 nm (mean diameter ~300 nm). PMMA-liposome system is more stable than licorice: for color stability, 2 to 5 times, for stability of glabridin content, 50 to 80%. Skin penetration of PMMA-liposome system is superior to that of general one by about 2 times. The reason of enhanced stability and penetration is that it is stabilized by NanoSolve and PMMA organized the skin structure materials.

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