# Lipid Nanospheres Containing Vitamin A and Vitamin E ; Their Stability and Release behaviors *In Vitro*

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## INTRODUCTION

Encapsulation techniques are used in pharmaceuticals, veterinary application, food, copying systems, laundry products, agricultural uses, pigments, and other less known uses to control the delivery of encapsulated agents as well as to protect those agents from environmental degradation. In particular, the science and technology of microencapsulation has potential uses in the pharmaceutical industry. In cosmetics, many health related items have been improved by micro-encapsulation techniques.[1]

In the present work, we try to deal with vitamin A and vitamin E as encapsulated materials. Vitamin A has a number of important functions in the body. It is necessary for growth and differentiation of epithelial tissue and required for the growth of bone, reproduction, and embryonic development.[2] Also, one of the important chemical features of the vitamin E (tocopherols) is that they are antioxidants. In acting as an antioxidant, vitamin E presumably prevents oxidation of essential celluar constituents or prevents the formation of toxic oxidation products. Especially, the vitamin E, tocopherols deteriorate slowly when exposed to air or ultra violet light. Therefore, to control the delivery rate of vitamin as well as to protect those agents from environmental degradation, we try to use encapsulation techniques.

Liposheres, which represent a new type of fat-based encapsulation technology developed for parenteral drug delivery, have also been used successfully as carriers of vaccines and adjuvants.[3] Liposheres consist of water dispersible, solid microparticles composed of a solid hydrophobic fat core, stabilized by one layer of phospholipid molecules embedded in their surface. Many studies of pharmaceutical devices have been carried out to develop suitable drug carrier for delivery using phospholipid. [4,5]

Since the physical stability of liposphere is also a problem of great importance (e. g., during employment and storage), much effort has been made in order to improve the in vitro and vivo stability.[6-8]

Aiming at encapsulation of fat soluble vitamin series, we prepared liposheres of soy bean lecithin coated with a nonionic surfactant and evaluated its in vitro stability. In addition, we report here some informations about the factor, which affect on the stability of liposhere. To evaluate the release properties from lipospheres, we carried out the permeability test *in vitro*.

## EXPERIMENTAL METHODS

### Materials

These vitamin A and E were supplied by Pacific Corporation R&D Center (Korea) and hydrogenated soybean phospholipids, supplied by Nikko Chemicals Co., Ltd.(Tokyo, Japan), contains about 30 % phosphatidylcholine and 70 % phosphatidyl-ethanolamine. All other ingredients were of reagent grade. Deionized-distilled water was used.

### Preparation of lipid nanosphere containing vitamin A and vitamin E

The surfactants such as soybean lecithin and the emulsifier adjuvant at a particular formula are added to fat soluble vitamin as an oil phase. Then, the mixture was dissolved in *n*-hexane. After the mixture was added dropletly into 2.25% glycerol solution, it was stirred with homogenizer at room temperature for 5 mins. The solution was followed by a vacuum evaporation at 30 ℃ to remove any residual *n*-hexane.

### Determination of particle size

To determine the mean size and size distribution of liposphere containing vitamin, dynamic light scattering (DLS) measurements were carried out using a dynamic light sacttering spectrometer (Model 95 ION Lager, Lexel Laser Inc., USA) at a wavelength of 514 ㎚ at 20 ℃. The intensity of the scattered light was detected at 90 ° to the incident beam. After the aqueous micellar solution was filtered with a microfilter having an average pore size of 0.8 ㎛ (Milipore, USA), size and size distribution were measured.

### Surface morpology of lipid nanosphere

The surface morpology of lipid nanosphere obtained by this procedure were examined with a scanning electron microscope. To prevent damage of lipid nanosphere by gold coating, we used FE-SEM apparatus without coating treatment. Also, we measured atomic force microscopy (AFM) to observe the surface morphology.

### Measurement of zeta-potential

The zeta-potential was calculated from the mean electrophoretic mobility of the oil droplets. Measurements of zeta-potential were performed in the same continuous phase.

### *In vitro* stability tests

After production, the lipid nanoparticle dispersions were stored at room temperature. During their storage, the mean size of the lipid nanoparticles was determined periodically by DLS measurement. From one bath, three samples were drawn and measured in triplicate.

### *In vitro* penetration study

The penetration experiment was performed in two chamber diffusion cells in vitro. Briefly, the cellulose membrane or rat skin included subcutaneous tissue were settled in between two chambers. The donor cell was filled with solution of lipid emulsion containing vitamin and receptor cell filled with release medium, then these apparatus was kept at 37 ℃. At predetermined time intervals, aliquots were withdrawn from the release medium. After the concentration of vitamin released was monitored using a UV spectrophotometer.

## RESULTS AND DISCUSSION

The lipid particle dispersion has a white, milky appearance. The lipid nanoparticles have almost spherical shape, as seen in the FE-SEM pictures in Fig. 1.

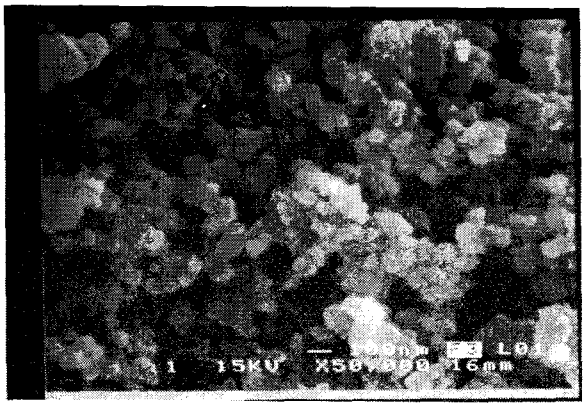
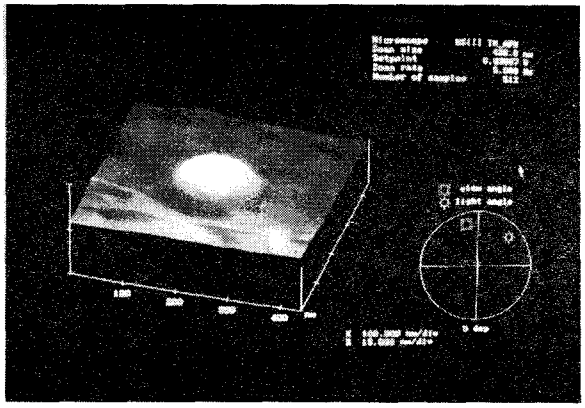


*Fig.1 FE-SEM image of lipospheres containing 20% of vitamin E*

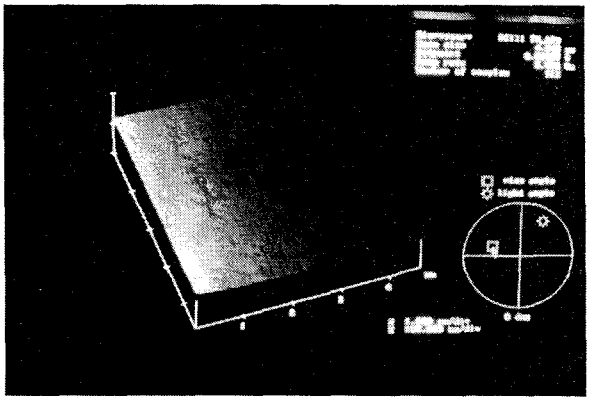
Also, the spherical shape of the lipid particles containing vitamin was evidenced by atomic force microscopy, as shown in Fig. 2. The mean size of the lipid nanoparticles is about 200nm range. This can be seen from the FE-SEM pictures (Fig. 1) and has been measured more accurately by DLS measurement. The size distribution of the typical sample measured by DLS was exhibited in Fig. 3. The sample was lipid nanospheres containing 2.5% oil phase emulsified by 1.2% lecithin. As shown in Fig. 3, sample showed the narrow and monodispersed distribution.

Lipospheres containing vitamin were prepared with fat soluble vitamin as an oil phase and soybean lecithin as a surfactant, and also an emulsifier adjuvant when necessary.

In this experiments, liposhere containing about 5-20% of vitamin A and E, respectively, can be prepared. The size and size distribution of liposhere were affected significantly by the component formulation such as vitamin contents, ratio of lecithin/vitamin, type of cosurfactants. Also, the stability of stored samples showed substantially distinguished characteristics between the sample of different formulations.



(a)



*(b)*

*Fig. 2 Images of lipospheres containing 5% of vitamin E taken by atomic force microscopy*

Emulsifying agents form good mechanical or electrical barriers to emulsion droplet coalescence. The surface potentials on the oil droplets play an important role in o/w emulsion stability through electrostatic repulsion. The change in zeta potential of oil droplets in the emulsions stabilized by soybean lecithins at various concentrations

was measured. Because of ionization of the various minor ingredients in lecithins such as phosphatidyl-ethanolamine, phosphatidylserine, phosphatidyl-inositol, the emulsion carries a negative charge at a pH value of around 7. The zeta potential decreases with the emulsifier concentration. These results show that an increase in the amount of negatively charged phospholipid (contained in the soybean lecithins) brings about a decrease in the zeta potential which recheaches a plateau region when the oil droplets in the emulsion are completely coated by the lecithin molecules. Therefore, the emulsion stabilized by 1.2 (w/w %) soybean lecithin are likely to be sufficiently stable with respect to these electrical barriers.

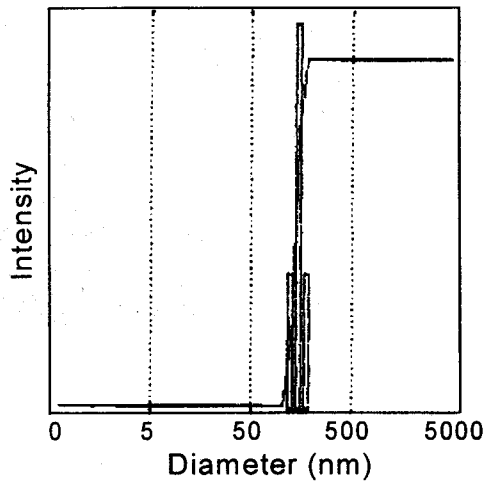



*Fig.3 Typical size distribution of lipid nanospheres containing vitamin E by dynamic light scattering measurement*

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