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# *In vitro* skin permeation of cubosomes containing triclosan

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ABSTRACT

Monoolein (MO) cubosomes containing triclosan (TCA) were prepared by micronizing MO cubic phases containing TCA in a bath-type sonicator. Pluronic F127 was used as a dispersant and no cubosomes could be produced from monoolein alone in the absence of the dispersant. The maximum tolerable ratio of TCA to MO for the formation of cubic phase was 16:84 (w/w). According to TEM photos and size distributions, the sizes of cubosomes were hundreds of nanometers. Using hydroxypropyl-β-cyclodextrin as a solubilizer for TCA, the *in vitro* skin permeation of TCA loaded in cubosomes was investigated on a diffusion cell. Even though the concentrations of TCA were the same in the suspensions of cubosomes, the skin permeations were increased more when the ratios of TCA to MO were higher in the cubosomes. Moreover, the permeations were higher than those of TCA suspension in aqueous solution of ethanol or propylene glycol.



## 1. Introduction

Lipid-based drug carriers have attracted much interest of scientists in the field of drug delivery system since they can envelop lipophilic active ingredients with a high efficiency and they are often known to enhance the efficacy of the ingredients. There are several kinds of lipidic carriers, including emulsions, liposomes, solid lipid nanoparticles, cubosomes and micelles. Among them, research on cubosome has been increasing due to the well-organized isotropic structures and the versatility as a drug carrier [1,2]. Cubosomes are nanoparticles of cubic phase and they can be prepared either by micronizing cubic phase in an excess aqueous phase [3,4] or dispersing dry lipid (e.g. monoolein) films into an aqueous phase [5]. Amphiphilic molecules, of which packing parameter is slightly greater than 1, are reported to form bicontinuous inverted cubic phase in an aqueous phase [6,7]. Monoolein (MO) is a representative amphiphile which could form the cubic phase. Two intercrossing water channels pass through the cubic phase and they are separated by MO bilayers. Hydrophilic compounds can be enveloped in the water channels and lipophilic one can be loaded in the bilayer. Due to its isotropic structure, the MO cubic phase is an optically transparent gel. Recently, insulin was entrapped in the water channel of cubosomes for the oral delivery [8,9]. The cubosomes were claimed to protect insulin from the harsh conditions of the stomach and to promote the gastrointestinal absorption of the hormone. Water soluble polymers such as alginate and poly (*N*-isopropylacrylamide) were loaded into the water channels to regulate the releases from cubosomes with respect to the change in multivalent ion concentration [10,11] and the change in temperature [12].

In this study, triclosan (TCA), a hydrophobic antibacterial agent, was loaded in cubosomes. The aims of the present work are to find out the maximum loading of TCA in MO cubic phase and to investigate the effect of the cubic phase nanoparticles on the *in vitro* skin permeation of TCA. MO cubic phases containing various amount of TCA were prepared by hydrating the molten MO/TCA mixture with an excess amount of distilled water at room temperature. Cubosomes containing TCA were prepared by micronizing the cubic phases using Pluronic F127 as a dispersant. Since TCA is widely used for the treatment of bacteria-related skin disease (e.g. acne), the efficacy of TCA-loaded cubosomes in the dermal delivery was evaluated through *in vitro* skin permeation experiments.

## 2. Experimental

### 2.1. Materials

Monoolein (1-monooleoyl glycerol, MO) was provided by Danisco Ingredients A/S (monoglyceride content is approximately 95.7% and oleic acid content is approx. 90%). Triclosan (TCA) was obtained from Ciba-Geigy ISP (Basel, Switzerland). Pluronic F127, hydroxypropyl-α-cyclodextrin (HP-α-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and phosphotungstic acid were purchased from Aldrich Chemical Co. (Milwaukee, USA). Water was distilled in a water purification system (Pure Power I$^+$, Human Corporation,

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Korea) until the resistivity was 18 MΩ/cm. All other reagents were in analytical grade.

### 2.2. Methods

#### 2.2.1. Preparations of cubic phases and cubosomes

1 g of MO and variable amount of TCA were put together into a vial. The ratios of TCA to MO were 0:100, 1:99, 2:98, 4:96, 8:92, 16:84, 24:76 and 32:68 (w/w). The solid mixtures were molten in a water bath, kept at 60 °C. Excess amount of distilled water (2.0 g), preheated at the same temperature, was added over the molten TCA/MO mixture and then it was kept at 25 °C until clear gels (cubic phase) were obtained. In order to obtain cubosome, 5 ml of Pluronic F127 solution in distilled water (30 mg/ml) was added to the MO/TCA gels so that the ratio of the polymeric surfactant to MO was 15:100. Pluronic F127 was used as a dispersant for the preparation of cubic phase nanoparticle (cubosome). And then, the gels were micronized in a bath type sonicator (VC 505, Sonic & Materials, USA, 30% energy intensity, 30 s pulse on, 30 s pulse off) at room temperature for 15 min. Pluronic F127 was used as a dispersant and no cubosomes could be produced from monoolein alone in the absence of the dispersant.

#### 2.2.2. Characterization of cubosomes

To observe the shapes of cubosomes, the suspensions of cubosomes were negatively stained with freshly prepared phosphotungstic acid solution (2%, pH 6.8) [13]. The stained cubosome suspensions were transferred onto a formvar/carbon coated grid (200 mesh) and it was air-dried at room temperature. The electron micrographs were taken on an electron microscope (LEO-912AB OMEGA, LEO, Germany). For the size measurements of cubosomes, MO concentration in cubosome suspensions was adjusted to 0.03%. The size of cubosome was determined on a particle size analyzer (ZetaPlus 90, Brookhaven Instrument Co., USA).

#### 2.2.3. In vitro permeation

The dorsal skins of female hairless mice (type SKH) aged 6 week were mounted onto Franz diffusion cells (0.636 $cm^2$ surface area) having 5 ml receptor compartment. Phosphate-buffered saline (PBS, pH 7.4) was used as the receptor content, thermostated to 37 °C under stirring. Cubosomes of which TCA to MO ratios were 4:96, 8:92 and 16:84 were used for the experiment of skin permeation. When the effect of TCA concentration in cubosome suspensions on the permeation was observed, the concentrations of MO in the suspensions of cubosomes were kept constant, 10 mg/ml. Accordingly, the concentration of TCA in the suspensions of the cubosomes of which TCA to MO ratios were 4:96, 8:92 and 16:84 were 0.41 mg/ml, 0.82 mg/ml, and 1.63 mg/ml, respectively. Even if the permeability of TCA through skin is high, the transfer from skin to receptor solution may be limited due to its poor solubility in the receptor solution. In order to increase the solubility of TCA in the receptor cell, HPCD solution in PBS (pH 7.4) was used to fill the receptor cell. To figure out how much HPCD should be included in the receptor cell solution to dissolve out TCA in the skin without suffering from the solubility limitation of TCA in the receptor solution, the solubility of TCA in PBS (pH 7.4) was determined with increasing the concentration of HP-α-CD or HP-β-CD, following a method described in a previous report [14–16]. On the other hand, when the effect of MO concentration in the suspensions of cubosomes on the permeation of TCA was observed, the concentrations of TCA in the suspensions of cubosomes (of which TCA to MO ratios were 4:96, 8:92, and 16:84) were kept constant, 0.41 mg/ml. 200 μl of the cubosome suspension was applied onto the skins and then the receptor solutions, 300 μl, were assayed for TCA using HPLC at the predetermined time. TCA suspension in aqueous solution of ethanol (1%) or propylene glycol (1%) was used as a control. The reason the concentrations of alcohols were adjusted to 1% in the controls is that the maximum concentration of MO in the cubosomes suspension were 1%. The TCA assay was performed in a liquid chromatograph (M600E, M7725i/Waters, 996PDA) equipped with a UV detector (0.05 AFUS) located at Central Laboratory of Kangwon National University. A reversed phase column (4.6 × 250 mm, C18 5 μm, XBridge™) was eluted with acetonitrile/water (75/25, v/v) at a flow rate of 1.0 ml/min and a sample of 20 μl was injected. The detection wave length was 280 nm.

## 3. Results and discussion

### 3.1. Preparation of cubic phases

Fig. 1 shows the photos of upside-down vials containing MO/TCA gels after hydration. All the preparations were stuck in the top of the vials, indicating that the gels were in a solid state. Transparent gels were obtained except when the ratios of TCA to MO were 24:76 and 32:68. Since cubic phase is known to be isotropic and optically transparent [4,17], the transparent gels are believed to be cubic phases. It means that TCA can be dissolved in cubic phase up to the TCA to MO ratio of 16:84 without disrupting the isotropic structure. Whereas, when the ratio of TCA to MO increased to 24:76 and 32:68, opaque white gel layer was formed over transparent gel layer. Because TCA is lipophilic and oil-soluble, the cubic phase would accommodate TCA in its hydrocarbon chain matrix. If so, the intercalation of TCA molecules between hydrocarbon chains of MO could occur, leading to an increased effective packing parameter of MO. In this circumstance, other phase rather than cubic phase is likely to be formed. It is believed that an inverted hexagonal phase is responsible for the opaque white gel layer [18].

### 3.2. Characterization of cubosomes

Fig. 2(a) shows TEM photos of cubosomes without TCA. The size was hundreds of nanometer, and blurred but some traces of water channels were observed within the cubosome particles. The cubosomes looked somewhat irregular on the TEM photo and they aggregated each other. This is due to a drying process during the preparation of replica. Hence, the shape on the TEM may somewhat different from the real one in the suspension. It was reported that the shapes on the cryo-TEM are square and rounded particles [3,19]. Fig. 2(b) shows TEM photo of cubosomes having TCA to MO ratio of 16:84, which was a maximum tolerable ratio without losing the transparency of cubic phases. When compared with cubosomes without TCA, no significant difference in the shape and the size were observed. It means that TCA can be dissolved in

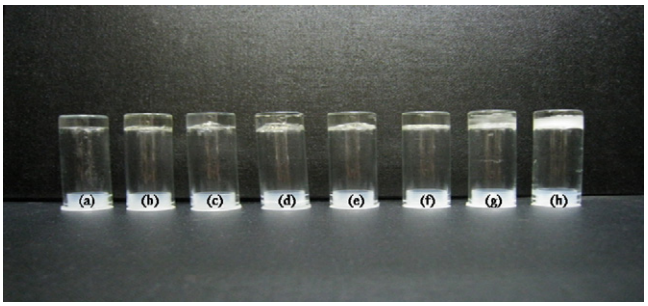


**Fig. 1.** The photos of TCA/MO gel in upside-down vials after hydration with excess amount of distilled water for 20 days at room temperature. The ratios of TCA to MO are 0:100 (a), 1:99 (b), 2:98 (c), 4:96 (d), 8:92 (e), 16:84 (f), 24:76 (g), and 32:68 (h) (w/w).