**ABSTRACT SUMMARY**

The purpose of this study was to encapsulate (+)-catechin (C) in non-ionic surfactant vesicles, namely niosomes in order to enhance drug delivery into the skin.

**INTRODUCTION**

(+) catechin (C) is one polyphenolic flavonoid constituent naturally present in green tea and other plants. This compound has gained great interest for its antioxidation effect and thus is considered to be a promising drug candidate in the fields of pharmaceutics and cosmetics. Numerous researches have focused on applying C topically in the aim of preventing harmful effects such as photoaging and skin cancers induced by exposure to UV radiation. How to overcome the skin barrier and improve the drug skin deposition is one big concern when C is tent to be applied topically. The main skin barrier is located within the stratum corneum (SC) which prohibits the passage of the majority of molecules. Various approaches have been used to overcome this barrier and increase the skin deposition such as the use of carrier systems including liposome and niosome.

Niosomes have a closed bilayer structure, formed from a self-assembly of nonionic surfactant(s) in an aqueous surrounding. Niosomes possess the advantages of low cost, high purity, good stability and are reported to be able to enhance drug penetration through SC and improve drug deposition.1 This type of carrier was invented and firstly brought onto market by Lancome®.

**EXPERIMENTAL METHODS**

Preparation of C-loaded niosomes. C-niosomes were prepared by thin film hydration method. Span 60 and cholesterol at a molar ratio of 4:1 with a certain amount of DCP (1.1mg) were dissolved in a mixture of chloroform and methanol (4:1, v/v) in a 50 mL round bottom flask and rotary-evaporated at 45°C until a thin, dry film formed on the wall of the flask (Buchi, Switzerland). The dried lipid film was purged with a stream of nitrogen for 5 min in order to get rid of residual traces of the organic solvent. The dry film was then hydrated with 15% ethanol in the water containing C at 60°C for 1 hr to form the drug loaded niosomes. The resultant niosomal suspension was set aside for at least 2 hr at room temperature to allow for the vesicle's membrane to anneal before being kept in a fridge. In order to obtain niosomes with homogeneous size, niosomes were sonicated for 3 min (Hielscher Ultrasound Technology, USA) and submitted to extrusion process for 10 cycles by an extruder device equipped with a 400 nm pore size polyester membrane.

Scanning electron microscopy (SEM). Niosomal dispersion was diluted with Mili-Q water and dropped onto the grid and dried under room temperature. Then they were sputter-coated with gold and palladium and examined for morphology with a Philips XL30S scanning electron microscope at 25kV.

Transmission electron microscopy (TEM). The size and morphology of niosome were also determined by negative staining-transmission electron microscopy. (FEI, USA).

Particle size and Zeta-potential (Zp). Niosomal vesicle size and potential were evaluated by dynamic light scattering using Zetasizer (Malvern Instrument, UK).

Determination of entrapment efficiency (EE). Ultracentrifugation was adopted in the separation of free and entrapped drug (Thermo Fischer Scientific Inc, USA). The precipitate of niosomes was dissolved in 20% Triton X-100 in Methanol. The drugs in the supernatant and precipitate were analyzed by HPLC to determine the encapsulation percentage.

In vitro release of C-niosome. Drug release profile was studied using Franz diffusion cell. The receptor chamber of diffusion cell was filled with 12ml of release buffer. Donor chamber was placed on top of the receptor with a cellulose membrane sandwiched in between the chambers. C-niosomal suspension and C solution were added in donor chamber. The diffusion cell was maintained at 37°C. 400µl aliquots were taken out from receptor compartment at defined time points and same amount of fresh release buffer was replaced.

Ex vivo skin permeation and deposition study. Percutaneous penetration and deposition studies were performed on Franz diffusion cell. The skin was sandwiched between the donor and receptor compartments with SC facing upwards into the donor. C-niosomal suspension and C solution were added in the donor. At different time points, 400µl aliquots were taken out from receptor compartment and analysed. The skin removed from the Franz-cell was wiped 10 times with methanol and the SC was removed by stripping with an adhesive tape for fifteen times. After tape-stripping, the skin was cut into small pieces and dissociated (Mitenyi Biotec Inc, USA). Then the dissociated sample was centrifuged and the supernatant was analyzed by HPLC.

**RESULTS AND DISCUSSION**

Size and Zp of C-niosomes. The size of vesicles influences their ability to act as drug delivery vehicles. The particle size of C-niosome was determined to be 204.0 ± 23.24 nm, with PDI of 0.272 ± 0.024 which was in accordance with the results of SEM and TEM observation. Niosomes exhibited negative Zp which was expected to be able to prevent particles from aggregating.

Entrapment efficiency. The EE of C-niosome was found to be 49.5 ± 1.3 %.

Release properties. From the release profiles, it appears that the diffusion of free drug from solution was fast and nearly complete (>90%) within 2 hours.
In comparison to the free drug solution, the efflux of C from the niosomal formulation was a biphasic process containing an initial fast phase followed by a sustained release phase. About 20% of C was released from niosomes over a period of 3 hours. The initial rapid release phase was followed by a prolonged release up to more than 20 hours and around 60% cumulative release of C at the end of 24h. The initial rapid release phase may be attributed by the permeation and desorption of free drug from the surface of niosomes and the sustained release phase relates to the diffusion of the drug through bilayers, which may leads to high retention of the drug. This kind of release pattern is of interest for dermal application in the view that the initial fast release improves drug penetration, while the further sustained release provides the drug over a prolonged period, which may maintain a therapeutic concentration in the skin without the need for frequent re-application.

Skin permeation and deposition study. The permeation profiles compare the amount of drug permeated through the skin with or without niosomal carrier during the period of 24h. The permeated drug amount was significantly ($p<0.05$) increased after 4 hours when niosomal formulation was used. This result might be due to the carrier effect of the niosome which is flexible because of the surfactant constituent to carry the drug pass through the SC. Also, vesicle can act as penetration enhancer to modify the structure of SC thus eases the penetration. When the drug was applied with niosomal carriers, the amount of drug retained in the skin was also enhanced, which may be attributed to the facilitated SC penetration and the controlled release property of C-loaded niosomes.

CONCLUSION

C-loaded niosomes were prepared by film-hydration method. The morphology, particle size, Zp, EE and drug release from its niosomal formulations were evaluated. The niosomal formulation was applied on the nude mouse skin and it was found that the application of niosomal formulation is able to enhance drug permeation and deposition.

REFERENCES