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**THE EFFECT OF PARTICLE SIZE OF ULTRADEFORMABLE LIPOSOMES FOR DERMAL DELIVERY OF HYDROPHILIC COMPOUND**

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**KEYWORDS:** Vesicle size, Ultradeformable liposomes (ULs), Hydrophilic compound, Skin permeation

**INTRODUCTION**

Ultradeformable liposomes consist of phospholipids, an edge activator that increases deformability of the bilayers by destabilizing them and are elastic in nature<sup>1</sup>. The influence of liposome size seems to be important. Verma et.al. (2003) reported that the hydrophilic fluorescent compound [carboxyfluorescein (CF)] penetration was related to the size of the liposome. The liposome with a size of 120 nm diameter showed statistically enhanced penetration of CF into the skin as compared to large ones<sup>2</sup>. However, no dedicated study was performed up to now to clarify the influence of vesicle size of ultradeformable liposomes (ULs) on the skin penetration. The aim of this study was to investigate the effect of vesicle size of ultradeformable liposomes (ULs) on the penetration of fluorescently labeled compound into the porcine skin. Sodium fluorescein (NaFI), a hydrophilic fluorescent compound was encapsulated into vesicles. Liposomes were prepared by the sonication method or extruding the vesicle through different pores sizes of membrane filter. The particle size, zeta potential, entrapment efficiency (%EE), Loading efficiency (%LE) and *in vitro* skin penetration were investigated. The confocal laser scanning microscopy (CLSM) was also used to investigate the penetration pathways of the vesicles.

**MATERIALS AND METHODS**

**Materials** Phosphatidylcholine (PC) from eggs was purchased from GmbH. Cholesterol (Chol) was purchased from Carlo Erba Reagent. Tween 20 was purchased from Ajax Finechem. NaFI was purchased from Sigma-Aldrich.

**Preparation of NaFI entrapped in liposome** Ultradeformable liposomes were composed of phospholipid (PC), cholesterol (Chol), and tween 20 as the edge activator. Liposomes were prepared by the sonication method or extruding the vesicle through different pores sizes of membrane filter. NaFI solution was prepared by dissolving NaFI in phosphate-buffered saline (PBS; pH 7.4). A mixture of PC and Chol in a molar ratio of 10: 2, dissolved in chloroform/methanol (2:1, v/v), was added to a test tube, and the solvent was evaporated with nitrogen gas. The lipid film was placed in a desiccator connected to a vacuum pump for a minimum of 6 hours. The dried lipid film was hydrated with NaFI in PBS buffer. After, the dispersion was sonicated in a bath for 30 minutes and then added tween 20 to the liposome dispersion. UL50 were prepared using probe-sonicated for 30 minutes. UL100 were prepared using probe-sonicated for 2 minutes and then extruding the vesicle through 0.22 µm membrane filter. UL 500 was prepared by extruding the vesicle through 1.2 µm membrane filter.

**Characterization of ultradeformable liposomes**

**Particle size and surface charge** Each liposome formulation was diluted with an appropriate amount of water and subsequently measured for size distribution and zeta potential using a Dynamic Light Scattering (DLS) particle size analyzer (Zetasizer Nano-ZS, Malvern Instrument, Worcestershire, UK) with a 4mW He-Ne laser at a scattering angle of 173°. All the measurements were carried out under ambient conditions and in triplicate.

**%EE and %LE** The liposome dispersion (0.5 mL) was placed in an ultrafiltration tube with a molecular weight cutoff of 3,000 Da (Microcon YM-3; Minipore, Billerica, Massachusetts, USA) and centrifuged at 4 °C at 10,000xg for 60 minutes. The filtration was discarded, and 0.25 mL of PBS was added to the retentate before further centrifugation at 4 °C at 10,000xg for 40 minutes. The collected NaFI-loaded liposome in the retentate were subsequently disrupted by added 0.2 mL of 0.1% (w/v) Triton X-100 and centrifuged at 4°C at 10,000xg for 10 minutes. The NaFI content in the supernatant was determined by the fluorescence-detection method. The drug %EE and %LE were calculated by the following equation (1 and 2)

$$\%EE = (C_L/C_i) \times 100 \quad (1)$$

Where C<sub>L</sub> is the concentration of NaFI-loaded in liposomal formulation, and C<sub>i</sub> is the initial concentration of NaFI added into the liposome formulation

$$\%LE = (D_i/L_i) \times 100 \quad (2)$$

Where  $D_i$  is the total amount of NaFI in the liposomal formulation, and  $L_i$  is the total amount of PC and Chol added into the liposomal formulation.

**In vitro skin-penetration study** NaFI through porcine skin was performed using Franz diffusion cells with a penetration area of  $2.31 \text{ cm}^2$ . The receiver compartment was filled with 6.5 mL of PBS (pH 7.4), stirred with a magnetic bar at a rate of 500 rpm. The skin was mounted in the diffusion chamber of the cell, with the stratum corneum side up. Diffusion cell were connected with a circulating water bath to maintain the temperature at  $32 \text{ }^\circ\text{C}$ . Each liposome (2 mL) was applied into the donor compartment. At the predetermined times of 1, 2, 4, 6, 8, and 24 hours, 0.5 mL of receiver medium was withdrawn for analysis by the fluorescence-detection method, and the same volume of PBS was added into the receiver compartment to maintain a constant volume. Each sample was analyzed in triplicate.

**CLSM study** After the in vitro skin-penetration study at a time of 8 hours, porcine skin was visualized the depth of NaFI penetration through the skin. The x-z sectioning confocal images were obtained from the x20 objective lens system, equipped with an He-Ar laser (excitation wavelength 488 nm; emission wavelength 514 nm). The piece of tissue was placed on a coverslip (22 x 50 mm) with the stratum corneum facing up toward the microscope condenser, than an adequate amount of methyl salicylate was added in to the piece of tissue as an immersion oil. Confocal images were illustrated as a gallery of the x-z axis serial optical sections.

## RESULTS AND DISCUSSION

**Physicochemical characteristic of ultradeformable liposomes** The particle size of ULs was in the order: UL50 ( $36.0 \pm 1.03 \text{ nm}$ ) < UL100 ( $120.83 \pm 1.21 \text{ nm}$ ) < UL500 ( $451.13 \pm 8.78 \text{ nm}$ ). Sonication is one of the popular methods to prepare unilamellar vesicles from the aqueous dispersion of phospholipids. In addition, using difference sonication times of an ultrasonic probe system have an impact on the liposome sizes<sup>7</sup>. All formulations showed a polydispersity index below 0.3 except for the 451 nm ULs, indicating a narrow size distribution. The zeta potentials of all formulations were negative surface charge ( $-14.93$  to  $-15.50 \text{ mV}$ ). (Table 1)

The %EE and %LE of NaFI-loaded ULs was in the order: UL500 > UL100 > UL50. It indicated that NaFI as a hydrophilic compound can be entrapped in the aqueous compartment (polar solute) of liposome vesicles. Since unilamellar vesicles contain a large central aqueous compartment, they are ideally suited for the encapsulation of water soluble agents<sup>8</sup>. In large unilamellar vesicles, UL500 showed higher NaFI-loaded liposome vesicle than UL50 and UL100.

Table 1 Characterization parameters of the difference liposomal formulations

Formulations	Particle size (nm)	PDI	Zeta potential (mV)	%EE	%LE
UL50	$36.13 \pm 1.03$	$0.199 \pm 0.01$	$-15.50 \pm 0.53$	$35.04 \pm 2.28$	$8.42 \pm 2.73$
UL100	$120.83 \pm 1.21$	$0.278 \pm 0.01$	$-13.20 \pm 0.56$	$36.79 \pm 9.00$	$10.17 \pm 2.89$
UL500	$451.13 \pm 8.78$	$0.367 \pm 0.01$	$-14.93 \pm 1.78$	$44.79 \pm 2.57$	$10.61 \pm 2.72$

All data represent the mean  $\pm$  standard deviation (n=3).

**In vitro skin-penetration study** The flux of NaFI can be ranged as follows:  $36 \text{ nm} > 138 \text{ nm} > 451 \text{ nm}$ . The smaller size ULs of 36 nm diameter showed enhanced penetration of NaFI in the skin as compared to larger ones. Fig.1 shows the amount of NaFI delivered from ULs of different sizes into different layers of the skin.

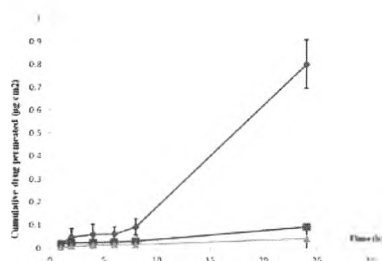
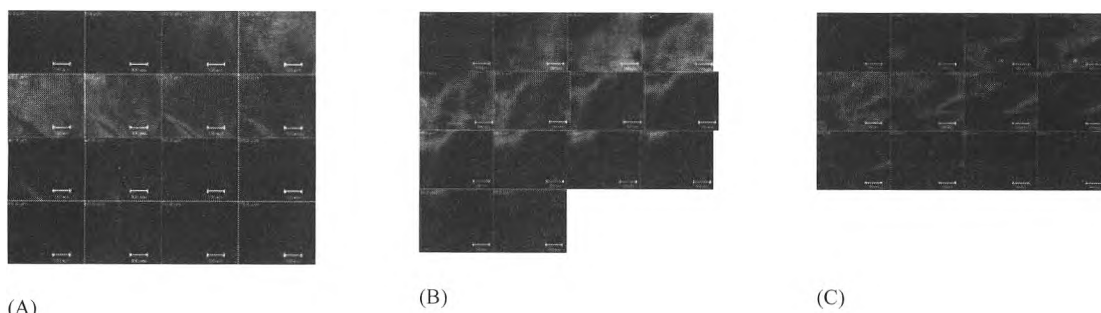


Figure 1 shows in vitro cumulative amount-time profiles of NaFI in difference formulations permeated through porcine skin. Symbols: UL 50 (●); UL100 (■); and UL500 (▲).

**CLSM study** CLSM study showed the difference in skin-penetration depth of NaFI between these three formulations. The smaller particle size (UL50) could penetrate into the deeper skin layer (75  $\mu\text{m}$ ) than UL100 (65  $\mu\text{m}$ ) and UL500 (55  $\mu\text{m}$ ).



**Figure 2** shows x-z axis serial images from CLSM of porcine skin treated with NaFI-loaded Rh-PE-labeled ULs at a time of 8 h: (A) UL 50; (B) UL 100; and (C) UL 500. (20x objective lens)

### CONCLUSION

The particle sizes were  $36.0 \pm 1.03$  nm,  $120.83 \pm 1.21$  nm, and  $451.13 \pm 8.78$  nm and had negative surface charge (-14.93 to -15.50 mV). The %EE and %LE of NaFI-loaded ULs was in the order: UL500 > UL100 > UL50. The flux of NaFI can be ranged as follows: UL50 > UL100 > UL500. The results correlated well with CLSM that the smaller size of vesicles showed the deeper skin permeation of NaFI. The transfollicular pathway also played an important role for skin permeation of ULs. The result indicated that the NaFI penetration was related to the size of the ultradeformable liposome.

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### REFERENCES

1. Cevc G., Schätzlein A., Richardsen H. 2002. Ultradeformable lipid vesicles can penetrate the skin and other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and direct size measurements. *Biochim Biophys Acta*, 1564(1), 21–30.
2. Verma D.D., Verma S., Blume G., Fahr A. 2003. Particle size of liposomes influences dermal delivery of substances into skin. *Int J Pharm*, 258, 141-151.
3. Subongkot T., Duangit S., Rojanarata T., Opanasopit P., Ngawhirunpat T. 2012. Ultradeformable liposomes with terpenes for delivery of hydrophilic compound. *J Lipo Res*, 22(3), 254-262.
4. Subongkot T., Wonglertnirant N., Songprakhon P., Rojanarata T., Opanasopit P., Ngawhirunpat T. 2013. Visualization of ultradeformable liposomes penetration pathways and their skin interaction by confocal laser scanning microscopy. *Int J Pharm*, 441, 151-161.
5. Kajimoto K, Yamamoto M, Watanabe M, Kigasawa K, Kanamura K, Harashima H, et al. 2011. Noninvasive and persistent transfollicular drug delivery system using a combination of liposomes and iontophoresis. *Int J Pharm*, 403(1–2):57–65.
6. Maheshwari RGS, Tekade RK, Sharma PA, Darwhekar G, Tyagi A, Patel RP, et al. 2012. Ethosomes and ultradeformable liposomes for transdermal delivery of clotrimazole: A comparative assessment. *Saudi Pharm J*, 20(2):161–70.
7. Silva R, Ferreira H, Little C, Cavaco-Paulo A. 2010. Effect of ultrasound parameters for unilamellar liposome preparation. *Ultrasonics Sonochemistry*, 17(3), 628–32.
8. Harrington KJ, Konstantinos N, Richard GV. 2002. Liposomal targeted cytotoxic drugs for the treatment of cancer. *J Pharm Pharmacol*, 54: 1573–1600.