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Silica-encapsualted Liposome Particles for High Loading and Release of Hydrophilic Niacinamide and Hydrophobic Retinol

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1. Introduction

Liposomes are microscopic spherical vesicle with the amphiphilic bilayers of hydrophilic and hydrophobic molecules. Liposomes have been widely used in various fields such as medical therapy and the formulation of pharmaceutical and cosmetic products due to their enhancement of the stability and entrapment of drugs or functional compounds into the hydrophobic layers [1,2]. In aqueous solution, liposomes have a very flexible bilayer structure that is fragile to external stimuli. The unstable nature of liquid liposomes causes great difficulty in the preparation and handling of medical and cosmetic formulations. Furthermore, the capacity for loading of drugs or functional compounds within the liposome bilayer is poor because the hydrophobic regions are too narrow [3]. To solve this problem, we recently reported several studies on the preparation of a controlled-release formulation of the solid, silica-coated liposome (silicified liposome) particles (SLPs) carrying natural compounds [4-6]. These reports only showed the application of silica liposome particles as sustained-release carriers, and that the release rate of drug and functional-cosmetic ingredients should be controlled by the silica layers. However, few studies have been conducted on the process of making reproducible silica-encapsulated liposome particles and few have investigated their cytotoxicity.

In this work, we reported the formation of spherical solid silica-encapsulated liposome particles (SLPs) under a variety of reaction conditions as functions of silica precursor concentration and reaction time, respectively. The phospholipid bilayer structure of the SLPs is confirmed by CLSM and FIB-SEM analysis, and the comparison of the compound-loading capacity of the solid SLPs for hydrophilic and hydrophobic compound (niacinamide and retinol, respectively).

2. Materials and Methods

Lecithin from soybean was purchased from Junsei Chemical (Seoul, Korea). The tetraethyl orthosilicate (TEOS, > 99%), the silica precursor, was purchased from Shinetsu (Seoul, Korea). Anhydrous ethyl alcohol (> 99%) was purchased from Daejung (Siheung, Korea). Fluorescein isothiocyanate (FITC) was purchased from Sigma Aldrich (Seoul, Korea). Preparation of SLPs

as a function of the TEOS concentration and reaction time was followed. Lecithin (1.5 g) was dissolved in 140 mL of ethanol and stirred at 350 rpm for 4 h. After centrifugation at 2000 rpm for 2 minutes, 70 mL of distilled water was dripped into the supernatant. Then, TEOS at each concentration (0.3, 0.45, and 0.6 M) was dripped into the solution at 35 °C, and kept for 4 h at 60 °C. Finally, the mixture solution was centrifuged at 3500 rpm for 10 min, washed with ethanol and distilled water (1:1), and then air-dried. To confirm the formation of SLPs as a function of reaction time, 0.3 M TEOS was dripped into the lecithin solution at 35 °C and then kept for 24, 36, 48, or 60 h at 60 °C, before testing. Moreover, we achieved reactions to form the SLPs at different temperatures (25, 60, and 80 °C). TEOS (0.3 M) was then dripped into the lecithin solution at 35 °C and then kept at 25 °C, 60 °C, or 80 °C. The morphological and structural details of the SLPs were studied by field emission scanning electron microscopy (FE-SEM), focused ion beam scanning electron microscopy (FIB-SEM), and transmission electron microscopy (TEM). FE-SEM investigations were carried out with a Tescan Mira-3 instrument using 2 kV of accelerating voltage. FIB-SEM analysis was carried out on a Thermo Scientific Helios G5 UC Dual beam using 2 kV of accelerating voltage. TEM was carried out on a JEOL JEM-2100 electron microscope operated at 200 kV. Confocal laser scanning microscope (CLSM) was carried out using a Carl Zeiss 980 with FITC-loaded in SLPs and liquid liposomes. Wide angle X-ray diffraction (WAXD) patterns were recorded using a Rigaku Miniflex 600 instrument with Cu-K_α radiation. The samples were scanned in the range 5° to 90° of 2θ with a scanning speed of 2°min^{-1}. Identification and characterization of the SLPs were carried out using a Fourier transform-infrared (FT-IR) spectrometer and thermogravimetric analysis (TGA). FT-IR spectra were measured using a Jasco 460 plus and the KBr compressed pellet method in the range 650-4000 cm^{-1}. Thermogravimetric analysis (TGA) was performed with a Q600 TA instrument at a rate of 10 °C min^{-1} in an N_2 gas from 25 °C to 700 °C. Measurement of the surface potential of the SLPs was performed using a zeta potential analyzer with ZEN 3600.

3. Results and Discussion

To elucidate the relationship between the formation of SLPs and the TEOS concentration, various properties of the SLPs were investigated as a function of the TEOS concentration. Figure 1(a) shows the spherical solid SLPs obtained by encapsulation with TEOS in the hydrophilic region of phospholipid bilayers by a sol-gel process are more robust and have better drug-loading efficiency than that of liquid liposomes**.** Figure 1(b) shows the correlation of particle size, surface potential, and thermogravimetric analysis of the SLPs as a function of the TEOS concentration. When the TEOS concentration increases to 0.6 M from 0.3 M, the size of an SLP was increases to 3.17 μm from 1.87 μm. This means that more silica fragments are encapsulated in the hydrophilic region of the SLPs particles, resulting in a thicker, and consequently, larger particle size. In fact, when the thickness of the SLPs was measured as the concentration of TEOS increased, it was confirmed that the thickness was increased to 0.18, 0.33, and 0.65 μm, corresponding to 0.3, 0.45, and 0.6 M TEOS, respectively. Accordingly, the particle size of the SLPs increased as the TEOS concentration increased, and the zeta potential showed a more negative value (as much as -12.07, -30.63, and -48.21 mV, corresponding to 0.3, 0.45, and 0.6 M TEOS, respectively). Thermogravimetric analysis was performed to determine the amount of lecithin in the SLPs with increase in the TEOS concentration. The amounts of lecithin slightly increased (within 80-85 wt.%) as the TEOS concentration increased. SEM images and the particle-size distribution of SLPs as a function of the TEOS concentration exhibit a spherical structure with increased particle size. Figure 1(c) shows the various properties of SLPs were investigated as a function of reaction time, up to 60 h from 24 h. As the reaction time increased to 24, 36, 48, and 60 h, the particle size of SLP particle size slightly increases to 2.09, 2.26, 2.42, and 2.50 μm, respectively. This is attributed to the notion that

the silica precursor was continuously condensed on the SLPs surface, which increased the particle size. Thickness of the SLPs as a function of reaction time was increased to 0.31, 0.43, 0.50, and 0.53 μm corresponding to 24, 36, 48, and 60 h, respectively. Furthermore, because the particle size of the SLPs increases as the reaction time increased, the zeta potential shows a more negative value (as much as -37.72, -39.82, -43, and -48.5 mV, corresponding to the 24, 36, 48, and 60 h, respectively). Thermogravimetric analysis was also performed to determine the amount of lecithin in the SLPs as the reaction time increased (the result was 76-81 wt.% of TEOS).

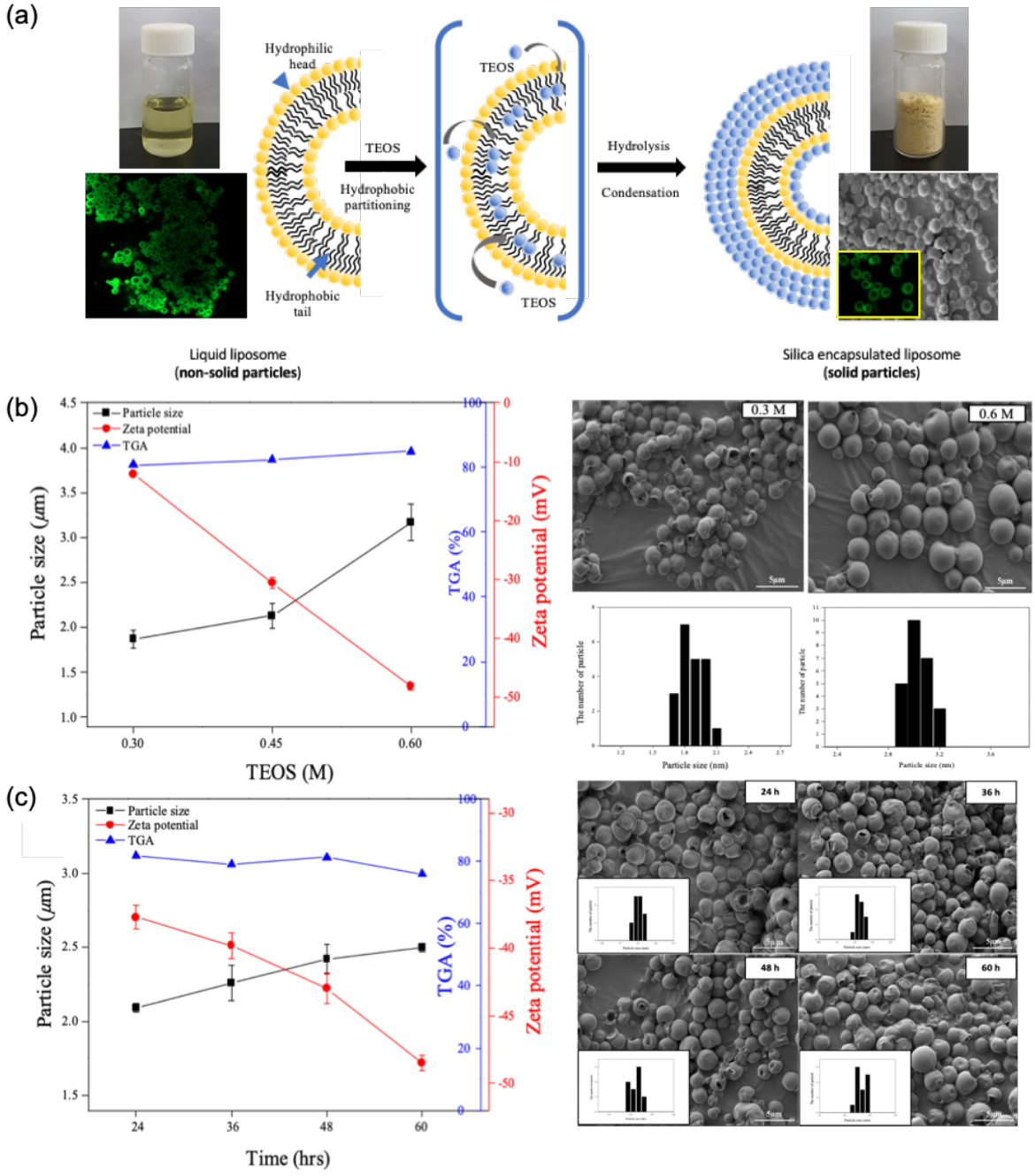

Figure 1. (a) Schematic representations of the silica-encapsulation in a liquid liposome by a sol-gel process. Correlation of particle size, zeta potential, TGA analysis, and SEM images of the SLPs as functions of (b) TEOS concentration and (c) reaction time, respectively.

Figure 2 shows the CLSM images of liquid liposomes and solid SLPs labeled with green-fluorescent FITC dye. The CLSM image shows the aggregated fluorescent ring structure of spherical bilayers of a liposome. The sol-gel silica encapsulation resulted in a yellow solid powder, as shown in the image. The confocal image shows more dispersed the SLPs with green-fluorescent intensity than in a liquid liposome. Moreover, we performed tomographic analysis of solid SLPs using a FIB-SEM, which confirmed the presence of a solid, hollow silica shell for the solid SLPs. The focused ion-beam scanning-electron microscope (FIB-SEM) images of the solid SLPs, in which the hollow structure and silica encapsulated layer are shown to include a lecithin layer. The silica encapsulation resulted in a thick and robust silica layer over the lecithin layer. The thickness of the silica layer and lecithin layer appear to be 0.5-0.7 μm, and 0.05-0.08 μm, respectively.

Figure 2. Confocal microscope images of liquid liposome particles (left, (scale bar is 5 μm), and FIB-SEM images of solid SLPs (right, scale bar is 1 μm).

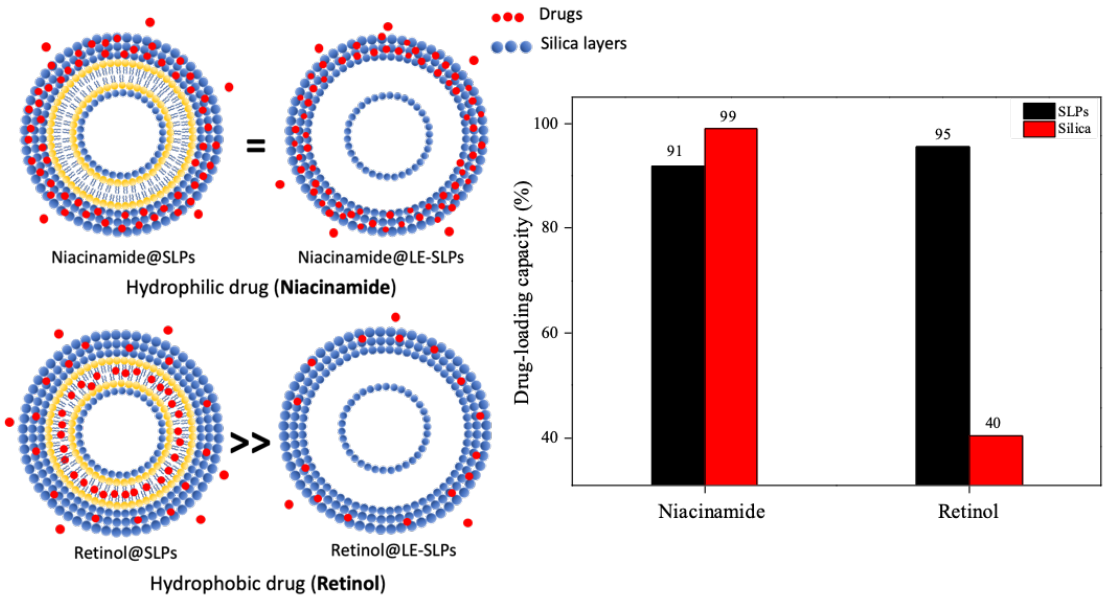

Figure 3. Comparison of the drug-loading capacity between the solid SLPs and liposome-eliminated SLPs with niacinamide, and retinol compounds, and comparison of the drug-loading efficiency of hydrophilic niacinamide and hydrophobic retinol in solid SLPs and LE-SLPs.

Figure 3 shows the comparison of the compound-loading capacity of the solid SLPs for hydrophilic and hydrophobic compounds (niacinamide and retinol, respectively). It is well known that the phospholipid bilayers in a liposome more easily entrapped a hydrophobic drug than hydrophilic drugs due to the hydrophobic partitioning effect. The solid SLPs showed the good drug capacity of 92, and 96 %, but, the liposome-eliminated SLPs showed the different compound capacity (99 and 40 %), for niacinamide, and retinol, respectively. This is because the hydrophilic drugs were well loaded in the hydrophilic silica layers and the hydrophobic drugs were well loaded in the hydrophobic phospholipid layers. Consequently, hydrophilic niacinamide was well loaded in both solid SLPs and LE-SLPs due to their silica layers, but hydrophobic retinol was well loaded in solid SLPs (but not in LE-SLPs) due to the phospholipid layers.

4. Conclusion

Spherical SLPs were successfully prepared by a sol-gel reaction of TEOS in the hydrophilic region of lecithin liposomes. Generally, the sizes of SLP particles were in the range 0.99 to 3.17 μm as a function of each reaction conditions. Moreover, the bilayer structure of the SLPs was confirmed by CLSM and FIB-SEM analysis. The compound loading capacity of the an SLP was > 95%, and in particular, the hydrophobic compound loading capacity was 2.3 times higher than that of general liposomes.

5. References

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