Encapsulation of the Retinal using the Charged Lipid Nano Particles (LNPs)

Poster ID 160

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Introduction:

Liposomes are lipid bilayer structure by the self-assembly of lipids. Liposomes are a spherical vesicle and most often composed of phospholipids. Liposomes have been used to encapsulate bioactive materials without chemical bonding, which can protect chemically sensitive molecules. However, the liposomes are vulnerable to leakage and aggregation due to hydrolysis and oxidation of lipids. Lipid nanoparticles (LNPs) are also formed by self-assembling process in lipid and aqueous environment [1,2]. LNPs have been applied to pharmaceutical industry for vaccine delivery such as mRNA vaccine [3,4]. LNPs have the hydrophilic and hydrophobic components, which could be encapsulated with various active ingredients. The physical and chemical stability of LNPs is more stable compared to the liposomes. In general, LNPs are composed of neutral phospholipids, cholesterol to enhance the stability of lipid membrane, and the negative lipids or the positive lipids. The charged lipids could be modified the structure of LNPs and surface characters [1]. LNPs take advantages that enhancing the solubility, augment the permeability, bioavailability, and protection of the active ingredient. LNPs have been manufactured by various method such as microfluidics, thin film hydration, reverse-phase evaporation, and emulsion method. Among them, the microfluidic systems have been recently applied. The microfluidics uses the microchannels to control the aqueous phase and lipid phase and can change the conditions (flow rate, temperature, flow ratio) for optimization of LNPs. Microfluidics take the advantages of controlling the particle size, high reproducibility, and possible to continuous operation [5]. Retinoids are defined as vitamin A or are chemically related to it. One of them, retinal, which is a form oxidized retinol, have more effective anti-wrinkle than retinol and led to increase dermal collagen synthesis. In this study, LNPs were used to encapsulate the retinal which is unstable under the oxygen environment.

Materials & Methods:

The LNPs were synthesized by using sodium dilauramidoglutamide lysine as ionizable anionic lipid and as follow changing the flow rate and ratio. Lipid solution was used with Distearoylphosphatidylcholine (18:0 PC (DSPC), Avanti Polar Lipids, Inc, USA) as neutral lipid, sodium dilauramidoglutamide lysine (Pellicer® L-30, Asahi Kasei Fine chem Co., Ltd., Japan) as charged lipid, PEG-120 stearate, polyglyceryl-4 caprate (PEC-10K, KIHOBio Co., Ltd., Korea), cholesterol (ACB Yeast Cholesterol JSQI, Active Concepts, USA) as stabilizer, and ethanol (Ethanol 95, Korea Alcohol Industrial Co., Ltd., Korea) as solvent. Retinal (Retinal, Shaanxi Yuantai Biological Technology Co., Ltd., China) was dissolved in lipid solution. Aqueous solution was prepared with purified water. The flow rate ratio was changed with various conditions as shown table 1. The synthesized LNPs were investigated using dynamic light scattering (Photal ELS-Z, Otsuka Electronics Co., Japan), ultraviolet–visible (UV) spectrometer, and fourier-transform infrared spectroscopy (FT-IR), and turbiscan (TurbiscanLAB, Leanontech, France). The turbiscan analysis was conducted at 40℃ for 4 h 30 min to obtain 50 scans.

Table 1. Flow rate ratio and sample notation of LNPs

	Lipid Solution	Aqueous Solution	Sample Notation
Flow rate ratio	1	3	L1W3(RA)
	1	4	L1W4(RA)
	1	5	L1W5(RA)
	1	6	L1W6(RA)
	1	7	L1W7(RA)
	1	8	L1W8(RA)
	1	9	L1W9(RA)
	1	10	L1W10(RA)

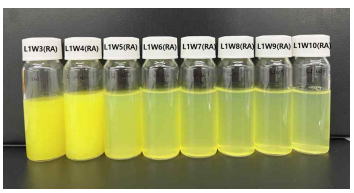

Figure 1. LNPs included the retinal

Results & Discussion:

The particle sizes of LNPs included the retinal were about 400 – 50,000 nm range. The zeta potential values of LNPs were all negative charge. The lipid and aqueous peaks were appeared in synthesized LNPs as shown in Fig. 4. The absorbance of retinal was shown at 360 – 370 nm in LNPs. Compared L1W6(RA) to L1W8(RA), the stability of L1W8(RA) was higher than others as shown in Fig.

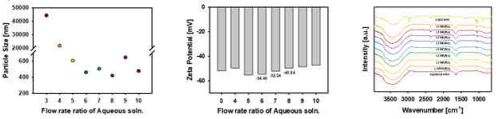

Figure 4. FT-IR Data of

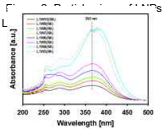

Figure 5. UV Data of LNPs

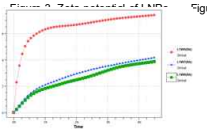

Figure 6. Stability of LNPs

The particle sizes were about 400 – 600 nm when the flow rate ratio of aqueous solution was 6 to 8. It could conclude that the L1W6(RA) – L1W8(RA) were successfully synthesized to LNPs. The synthesized LNPs were the negative surface charge. Among them, L1W6(RA) – L1W8(RA) samples were higher negative charge, which could conclude that they were more stable than others. Fig. 4 shows the chemical stretch bonds of LNPs included retinal. The flow rate ratio of aqueous solution was increased, the intensity of peaks related lipid was decreased. The maximum absorbance of LNPs was about 360 nm, which means synthesized LNPs were successfully encapsulated the retinal as shown in Fig. 5. But, L1W3(RA) and L1W4(RA) were shown that the retinal didn't be encapsulated as shown in Fig.1, 2. The stability of LNPs were shown in Fig. 6. TSI is new features called as stability index of turbiscan analysis, that the lower index is stable. The L1W8(RA) was more stable than the others.

Conclusions:

The LNPs included the retinal were synthesized by microfluidics. The particle size of LNPs were range of 400-600 nm and zeta potential were all negative charge. The LNPs encapsulated the retinal were successfully synthesized and stability enhanced. The further study will be conducted to enhance the stability. This study could apply for various fields such as drug delivery, food, and cosmetics.

Acknowledgements:

This work was supported by the Technology Innovation Program (or Industrial Strategic Technology Development Program (10077704, Development of skin-sensitized organic-inorganic hybrid with improved skin penetration for functional cosmetics) funded By the Ministry of Trade, Industry &Energy (MOTIE, Korea).

References:

1. P. Kalyanram, A. Puri, A. Gupta (2022) Thermotropic effects of PEGylated lipids on the stability of HPPH-encapsulated lipid nanoparticles (LNP). J Therm Anal Calorim 147:6337-6348
2. R. Tenchov, R. Bird, A.E. Curtze, Q. Zhou (2021) Lipid Nanoparticles From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement. ACS Nano 15:16982-17015
3. B. N. Aldosari, I. M. Alfagih, A. S. Almurshedi (2021) Lipid Nanoparticles as Delivery Systems for RNA-Based Vaccines. Pharmaceutics 13:206
4. A. M Reichmuth, M. A Oberli, A. Jaklenec, R. Langer, D. Blankschtein (2016) mRNA vaccine delivery using lipid nanoparticles. Ther Deliv 7: 319-334
5. M. Maeki, S. Uno, A. Niwa, Y. Okada, M. Tokeshia (2022) Microfluidic technologies and devices for lipid nanoparticle-based RNA delivery. J Control Release 334: 80-96