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"Stabilization of Retinal using Poly(2-oxazoline) based Polymer-Lipid Hybrid Nanoparticle"

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1. Introduction

Poly(2-oxazoline) (POx) is a polymer synthesized via the ring-opening polymerization of 2-oxazoline monomers. It is considered a biomimic polymer or pseudopeptide due to its structural relationship to polypeptides [1-2]. POx is characterized by its non-ionic nature, good biocompatibility, excellent chemical stability, and high solubility in water and various organic solvents, which has led to its wide application in various fields such as drug delivery systems and biomaterials [3]. Furthermore, POx has been identified as a potentially valuable in cosmetic applications, as its hydrophilicity and hydrophobicity can be precisely modulated by modifying the substituents, and its peptide-mimicking structure provides strong adhesion properties to the skin [4].

POx is known to generate carboxylic acid derivatives as degradation by-products during hydrolysis, whereas Polyethylene Glycol (PEG) is known to generate toxic 1,4-dioxane, one of the ingredients to be avoided in cosmetics during hydrolysis [5]. Despite the widespread use of PEG in drug delivery systems, adverse effects such as the presence of PEG antibodies in the blood have been reported, and the non-biodegradability of PEG results in the accumulation of high molecular mass PEG in the body. In contrast, POx has garnered attention as a substitute for PEG due to its low immunogenicity and excellent biocompatibility [6-7]. POx is promising polymer for drug delivery systems and as a cosmetic ingredient, as it is relatively easy to synthesize and presents not only high chemical stability but also good cytocompatibility and hemocompatibility, biodegradability, and low in vitro toxicity [4].

Retinal is a vitamin A derivative, a compound that has functional similarities to vitamin A and is widely used in cosmetics [8]. Retinal is considered to be one of the most effective anti-aging ingredients, improving skin texture, dyspigmentation, dryness and fine lines. However, due to its physicochemical properties, Retinal is very unstable and degrades rapidly under the influence of light, oxygen, metal ions, and oxidizing agents, as well as when subjected to

elevated temperatures [9]. In this study, we aim to stabilize the unstable Retinal by protecting it. The Retinal stabilization is achieved by encapsulating it in an amphiphilic polymer, a POx-PCL-based polymer-lipid hybrid nanostructure.

2. Materials and Methods

2.1. Synthesis of the POx-PCL

The synthesis of POx-PCL is initiated by creating an inert atmosphere using argon gas. POx is dissolved in chlorobenzene at 140 °C. Then, caprolactone and stannous octoate are introduced into the solution and stirred at 140 °C for a for 48 hours. After this, the resultant POx-PCL is precipitated in diethyl ether, and the precipitate is separated and dried in a vacuum oven at 40 °C.

2.1. Synthesis of the Polymer-Lipid Hybrid Nanoparticle(PLHN)

The synthesis of POx-PCL-based PLHN was carried out according to the following procedure. Initially, heated phase A (75 – 80 ℃) was slowly added to heated phase B (80 ℃), and emulsification was carried out using a mixer for 1-2minutes. Subsequently, phase C (50 – 60 ℃) was slowly added to the solution and emulsified using a mixer for 5minutes. Finally, the PLHN is synthesized using a microfluidizer(Table 1).

Phase	Ingredient	% by weight	
		PLHN	General Liposome
A	Hydrogenated Lecithin	2.00	2.00
	Water	69.98	70.00
	Glycerin	20.00	20.00
	1,2-Hexanediol	2.00	2.00
	POx-PCL	0.02	-
B	Retinal	1.00	1.00
	Caprylic/Capric Triglyceride	5.00	5.00

Table 1. Prescription of PLHN and General Liposome Formulation

3. Results

3.1. Characterization of the POx-PCL

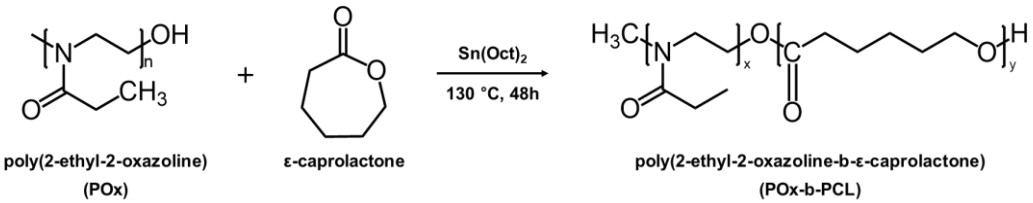
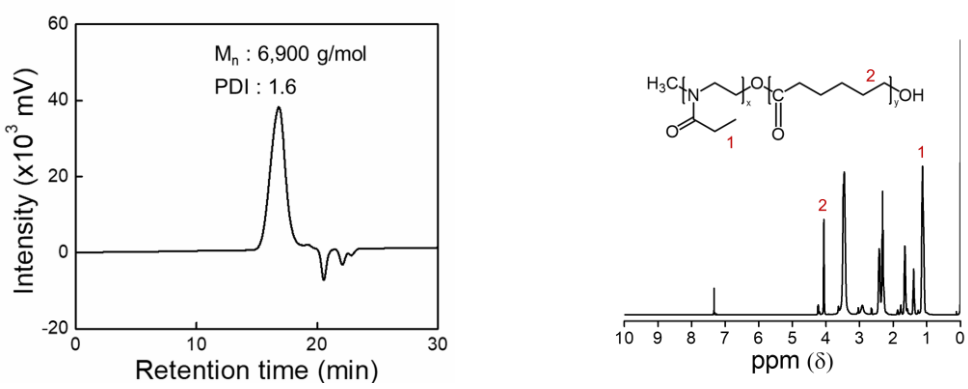


Figure 1. POx-PCL Synthesis schematic

POx-PCL was synthesized by ring-opening polymerization of ε-caprolactone, as shown in the schematic diagram in Figure 1. The resulting POx-PCL is an amphiphilic polymer composed of hydrophilic POx and hydrophobic PCL (Figure 1).



(a) **(b)**

Figure 2. Characterization of POx-PCL. (a) Molecular weight of the synthesized POx-PCL; (b) NMR of the synthesized POx-PCL.

To confirm the synthesis of POx-PCL, an analysis of the molecular weight and structure was conducted. The molecular weight of the synthesized POx-PCL was measured to be 6,900 g/mol, with a PDI value of 1.6 (Figure 2 (a)). In the NMR analysis, the characteristic peak of POx, the hydrophilic block, was observed at about 1 ppm and the characteristic peak of PCL, the hydrophobic block, was observed at about 4 ppm(Figure 2 (b)). The presence of the characteristic peaks both POx and PCL in the synthesized samples , thus confirming the successful synthesis of the POx-PCL block copolymer.

3.2. Self-assembly and emulsification of the POx-PCL

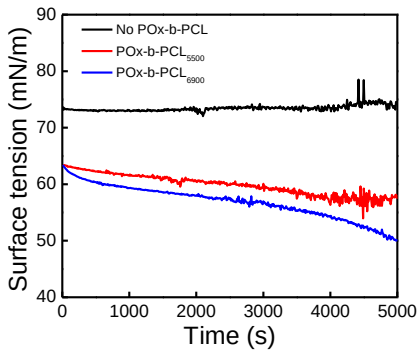


Figure 3. Surface Tension of POx-PCL

The capacity for emulsification of POx-PCL was confirmed by surface tension measurements. In the absence of POx-PCL, the surface tension of purified water was found to be constant between 70 and 75 mN/m. However, when POx-PCL (MW: 6,900 g/mol) was present, the surface tension was measured to be less than 60 mN/m, indicating a strong interfacial effect. The application of the synthesized POx-PCL resulted in a reduction in surface tension, which demonstrates the POx-PCL has emulsifying properties (Figure 3.).

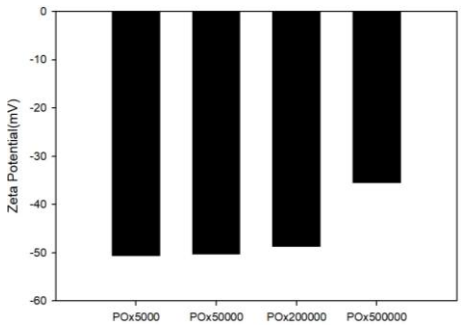
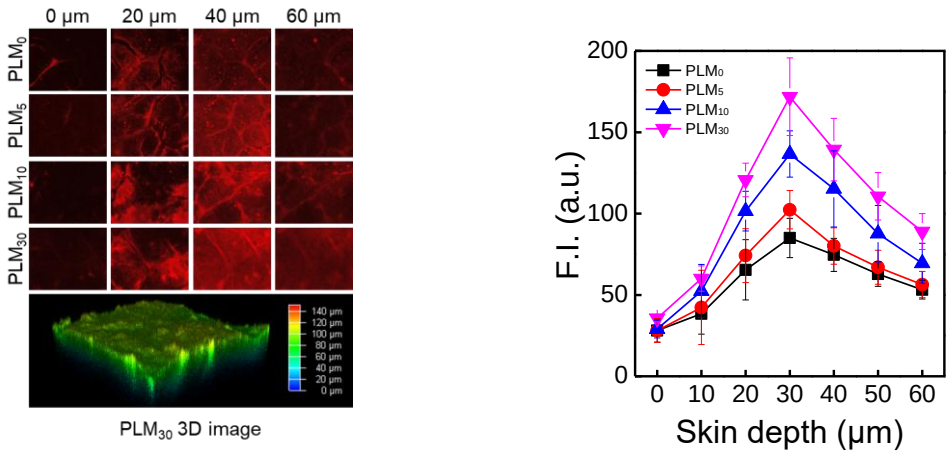


Figure 4. Zeta Potential of PLM by POx molecular weight

We measured the zeta potential of polymer-lipid micelle (PLM) as a function of POx molecular weight. PLM prepared with POx of 5 kDa, 50 kDa, 200 kDa, and 500kDa exhibitied zeta potentials of -50.76 mv, -50.33 mV, -48.79mV, and -35.52 mV, respectively (Figure 4). These data reveal an inverse relationship between POx molecular weight and the magnitude of negative surface charge: lower-mass POx yields PLM with greater absolute zeta potentials. Hence, PLM formulated with lower-molecular-weight POx demonstrate enhanced colloidal stability.



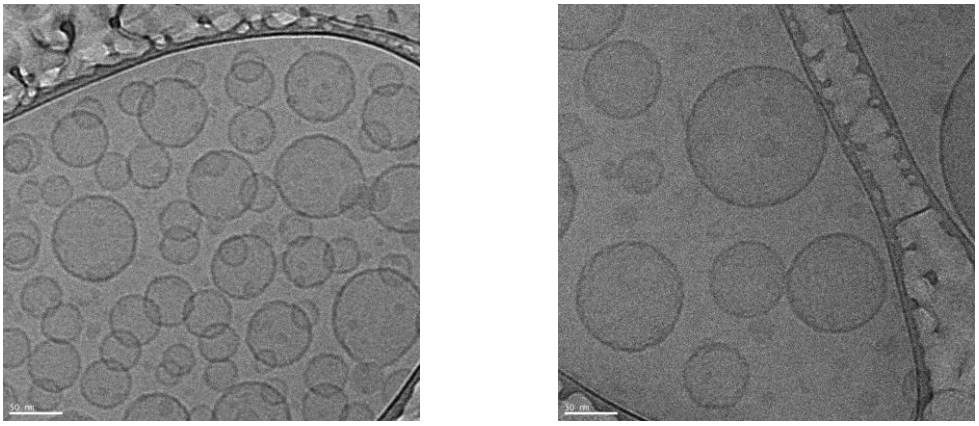
(a) **(b)**

Figure 5. Percutaneous absorption of PLM containing varying POx-PCL ratios. (a) CLSM images showing skin penetration; (b) Quantitative penetration profiles at incremental skin depths.

We performed an analysis of PLM percutaneous absorption varying POx-PCL ratios. CLSM images and quantitative data revealed peak penetration at 30 μm for all formulations. Moreover,

penetration efficiency increased in the order PLM_0 < PLM_5 < PLM_{10} < PLM_{30} at every depth tested, with PLM_{30} achieving approximately double the penetration of PLM_0 at 30 μm. These results confirm a positive correlation between POx-PCL content and skin absorption (Figure 5 (a) and (b)).

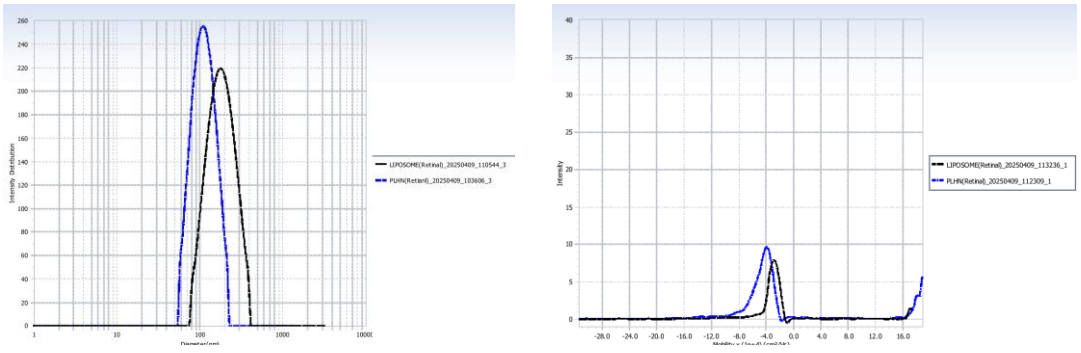
3.3. A comparison of the retinal-loaded PLHN and retinal-loaded general liposome



(a) **(b)**

Figure 6. cryo-TEM analysis of PLHN and General Liposome. (a) PLHN; (b) General Liposome.

We examined PLHN morphology by cryo-TEM and confirmed the formation of nanosturctures. Compared with the general liposome control, PLHN exhibited significantly smaller nanostructures (Figure 6 (a) and (b)).



(a) **(b)**

Figure 7. Characterization of PLHN and general liposome. (a) Particle size of the PLHN(blue) and general liposome(black); (b) Zeta potential of the PLHN(blue) and general lipsome(black).

Retinal was encapsulated in the synthesized POx-PCL based PLHN and the particle size and zeta potential were measured. The results obtained indicated a particle size of 98.1 nm, PDI value of 0.121 and a zeta potential of -53.50 mV. In contrast, the particle size of the general

liposome encapsulating retinal was measured at 176.5 nm, PDI value of 0.243 and a zeta potential of -36.80 mV (Figure 7 (a) and (b)).

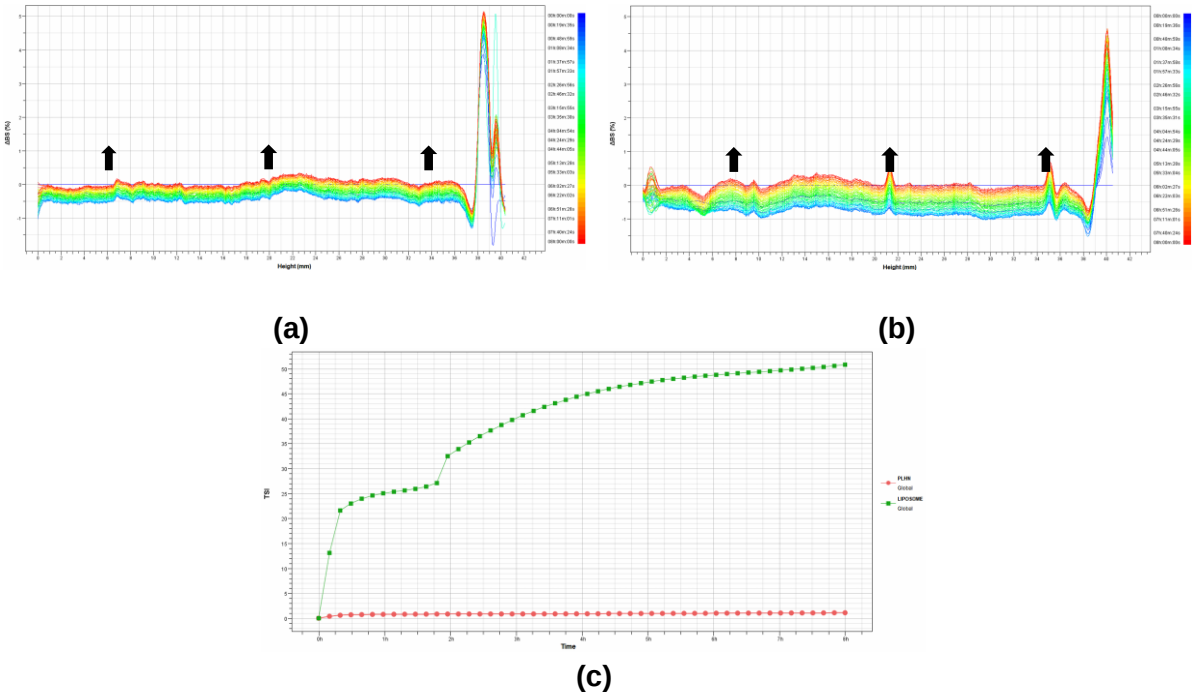
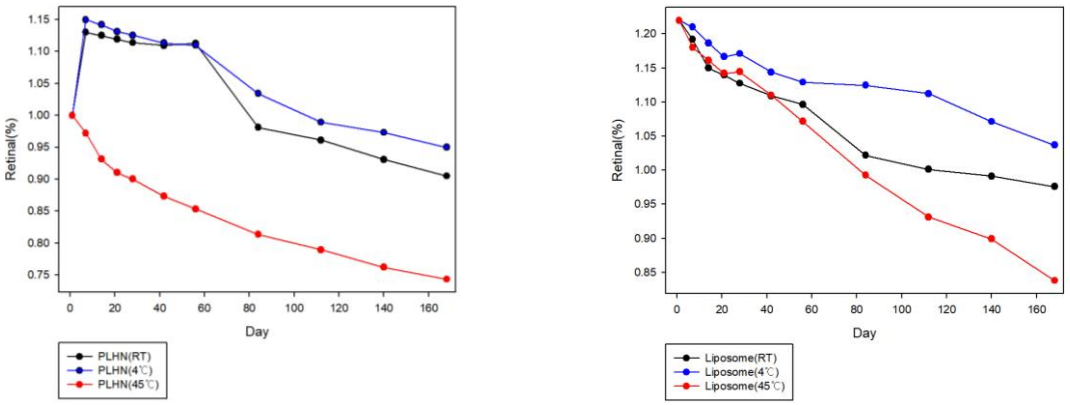


Figure 8. Terbiscan Data of PLHN and general liposome. (a) Delta backscattering of PLHN; (b) Delta backscattering of general iposome; (c) Turbiscan data TSI of PLHN and general iposome

The dispersion stability of the samples over time was measured using the Multiple Light Scattering Method. The results demonstrate an increase in the backscattering (BS) (%) value for both samples over time, indicating that particle size changes have occurred due to flocculation and coalescence. PLHN exhibited less variation in the measured BS (%) values compared to general liposome, indicating a more stable dispersion (Figure 8(a) and 5(b)). The TSI (Turbiscan Stability Index, Global), which reflects the change in dispersion stability over time, indicates that higher TSI values correspond to poorer stability. A comparison of the TSI values for PLHN and general liposome shows that PLHN has a lower TSI value than general liposome, confirming that PLHN is relatively more stable (Figure 8(c)).



(a) **(b)**

Figure 9. HPLC Data of Retinal-loaded PLHN and Retinal-loaded general liposome. (a) The retinal potency of PLHN at 25℃, 4℃, 45℃; (b) The retinal potency of general liposome at 25℃, 4℃, 45℃

The retinal titer stability of POx-PCL-based PLHN and conventional general liposome was evaluated over six months. PLHN retained 94%, 90%, and 76% of their initial retinal titer at 4 ℃, 25 ℃, and 45℃, respectively (Figure 9(a)). In contrast, general liposome retained 85%, 80%, and 70% of their retinal titer under the same conditions (Figure 9(b)). These results confirm that retinal encapsulated in POx-PCL-based PLHN exhibits superior long-term stability across all tested temperatures.

4. Discussion

The synthesis of hydrophilic POx and hydrophobic PCL to form POx-PCL was carried out via ring-opening polymerization, and the structure of the resulting block copolymer is shown in Figure 1. The molecular weight of POx-PCL was determined to be 6,900 g/mol, and NMR analysis confirmed the presence of characteristic peaks for bothe POx and PCL, indicating successful synthesis. The surface tension of POx-PCL was measured to be less than 60mN/m, suggesting that POx-PCL has the potential to function as an effective emulsifier. Zeta-potential measurements of PLM with varying POx molecular weights showed that PLM derived from lower-molecular-weight POx exhibited higher colloidal stability. Moreover, increasing the POx-PCL content enhanced transdermal absorption rates, indicating that incorporation of POx-PCL can improve PLHN stability.

POx-PCL-based PLHN was used to stabilize retinal, and the characteristics and stability of retinal-loaded PLHN and retinal-loaded general liposome were compared. TEM analysis revealed that the synthesized PLHN particles possess a well-defined nanostructure. The average particle size was measured to be 102.4nm, with a PDI value of 0.121 and zeta potential of -53.50 mV, indicating that the PLHN exhibited a high uniformity. The dispersion stability of the PLHN was evaluated using Turbiscan analysis, which revealed relatively low TSI values. This suggests that the PLHN exhibit better dispersion stability compared to lipopsome and are capable of forming stable emulsion. A comparison of retinal potency indicated that the PLHN

maintained higher potency stability across a range of temperatures, demonstrating that POx-PCL-based PLHN is effective for retinal stabilization. Consequently, POx-PCL-based PLHN has demonstrated excellent dispersibility and substantial retinal potency stability. These properties indicated their potential for application in cosmetics, particularly in formulations of cosmetics free of PEG derivatives.

5. Conclusion

In this study, amphiphilic POx-PCL was synthesized by conjugating hydrophobic PCL to the hydrophilic POx termini via ring-opening polymerization. The successful formation of POx-PCL was confirmed by NMR analysis. Subsequent surface tension measurements demonstrated the emulsifying properties of POx-PCL, which was the applied to stabilized retinal.

The POx-PCL-based PLHN was used for retinal stabilization, and the synthesized PLHN was found to exhibit a well-defined nanostructure and a uniform nanometer-scale size distribution. The dispersion stability of the synthesized PLHN was confirmed by Turbiscan analysis, and the retinal potency stability of the PLHN was confirmed by HPLC analysis. These results indicated that POx-PCL-based PLHN are effective for retinal stabilization. Our results show that POx-PCL-based PLHN have high dispersion stability and encapsulation efficiency compared with general liposome, indicating that PLHS is capable of stably encapsulating active ingredients. Therefore, POx-PCL can be used as an alternative to PEG derivatives, and POx-PCL-based PLHN has potential applications in various fields such as drug delivery systems, cosmetics and etc.

6. References

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