Preparation and Characterization of Nisin-conjugated Liposome as a Drug Delivery System

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Abstract

This research focuses on the preparation and characterization of liposomes conjugated with nisin peptide to enhance efficiency and specificity for targeted drug delivery applications. Nisin, a naturally occurring antimicrobial peptide with positive charges, was selected as a ligand for conjugation to improve specificity to cancer cells. Key properties of the liposomes, including particle size, stability, nisin conjugation efficiency, drug encapsulation efficiency, and drug release rate, were evaluated to demonstrate the suitability of the system for medical applications under simulated physiological conditions. This study aims to compare two bioconjugations methods for peptide and liposomes, including 1) carbodiimide conjugation and 2) thiol-maleimide methods. Liposomes were mainly composed of soy phosphatidylcholine and cholesterol and encapsulated with doxorubicin via the remote loading method. Particle size of non-conjugated liposomes analyzed using dynamic light scattering (DLS) were in the range of 120-130 nm and increased after conjugation. Nisin-conjugated liposomes via both methods exhibited slightly negative values of zeta potential, suggesting tendency of aggregation. Drug release of liposomes was analyzed over 15 days using the dialysis method under optimized conditions and fitted into Korsmeyer-Peppas kinetic model. Nisin conjugation efficiency and drug encapsulation efficiency were comparable, but the drug release rate was lower in thiol-maleimide conjugation method. Thus, the thiol-maleimide conjugation at 1:20 ratio was chosen as overall it demonstrated superior performance in terms of physicochemical properties and drug release. However, conditions need to be further optimized for the stability and long-term release behavior in the delivery system.

Introduction

- **Liposomes** are nanometer-scale structures composed of a phospholipid bilayer surrounding an aqueous core. They serve as highly effective drug delivery carriers due to their ability to enhance the solubility of poorly water-soluble drugs, protect active compounds from degradation, and improve drug absorption into target cells.
- **Nisin A** is a 34-amino acid peptide produced by *Lactococcus lactis* and belongs to the class of positively charged antimicrobial peptides known as lantibiotics, specifically type A lantibiotics. It exhibits potent antibacterial activity against Gram-positive bacteria.
- Incorporating nisin into a liposomal drug delivery system presents a promising approach to improve its stability and target specificity. This strategy could potentially increase the efficacy of nisin while extending its circulation time.

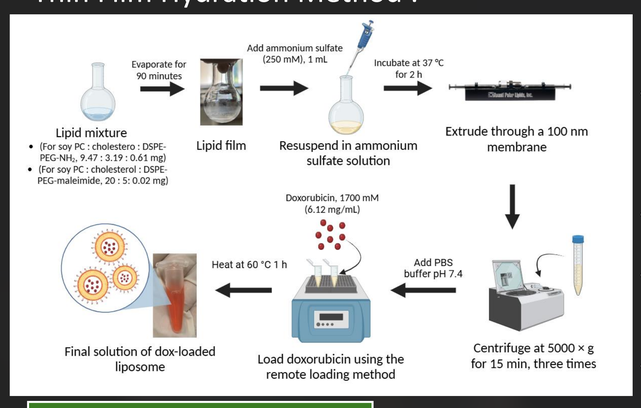
Objectives

- To investigate the physicochemical properties by comparing two peptide conjugation methods, carbodiimide cross-linker conjugation and thiol-maleimide conjugation, aiming to enhance specificity and efficiency in drug delivery.
- To study the optimal drug release rate for targeted drug delivery and its suitability for medical applications

Methodology

Lipid preparation

Thin Film Hydration Method :

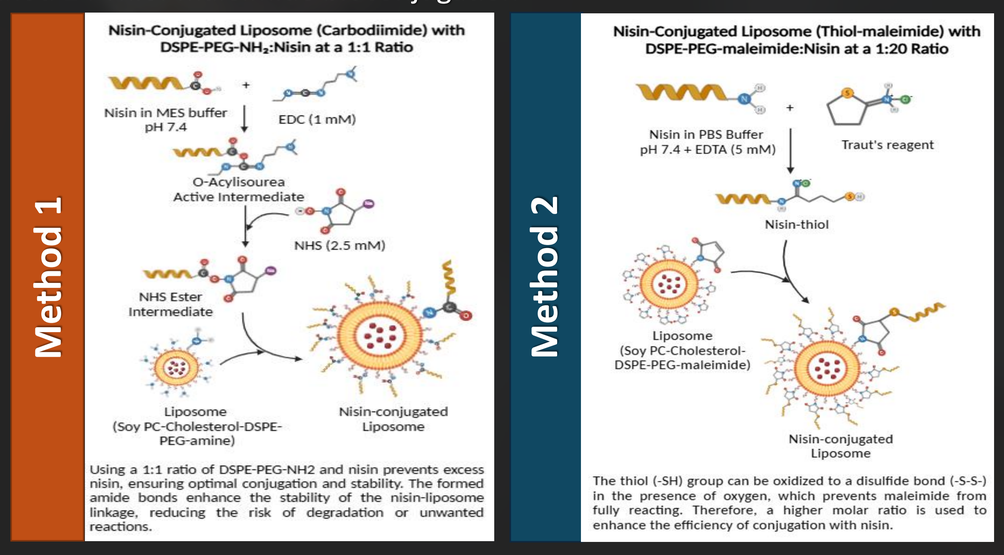


- For **Carbodiimide cross-linker bioconjugation**, the liposome is composed of soy PC+ cholesterol + DSPE-PEG-amine in a 56:38:1 ratio, with amounts of 9.47, 3.19, and 0.61 mg, respectively.
- For **Thiol-maleimide bioconjugation**, the liposome is composed of soy PC + cholesterol +DSPE-PEG-maleimide in a 40:20:0.01 ratio, with amounts of 20, 5, and 0.02 mg, respectively.
- Quantification of phospholipids by Phosphorus-assay.
- The drug encapsulation efficiency (%EE) was analyzed by measuring absorbance at 480 nm using a microplate reader.
- Size, zeta potential, and PDI were analyzed using dynamic light scattering (DLS).

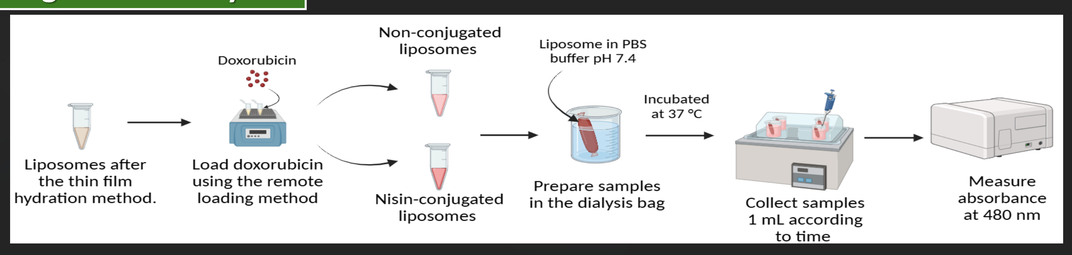
Bioconjugation methods

Method 1 : Carbodiimide cross-linker bioconjugation

Method 2 : Thiol-maleimide bioconjugation



Drug release analysis



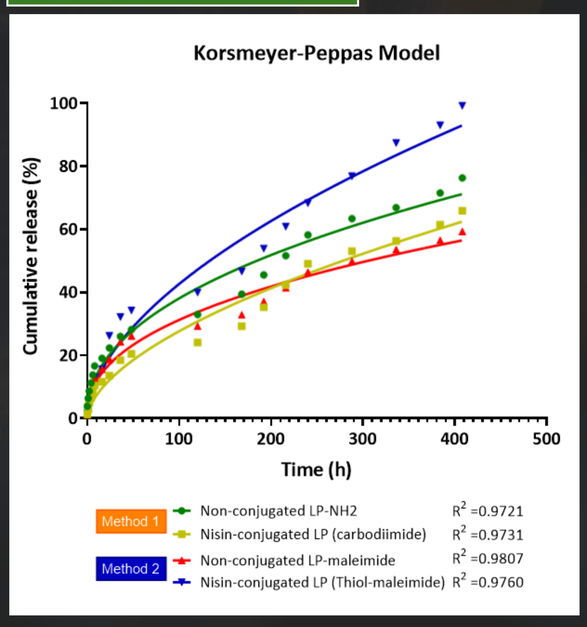
Results and Discussion

Physicochemical Characterization of Liposomes

	Liposomes	Size (nm)	PDI	Zeta Potential (mV)	%Nisin conjugation	%EE
Method 1	Non-conjugated liposome (LP-DSPE-PEG-NH2)	131.40 ± 2.36	0.156 ± 0.049	5.30 ± 7.96	-	98.83 ± 0.139
	Nisin-conjugated liposome (carbodiimide), LP:NS ratio 1:1	228.20 ± 12.59	0.654 ± 0.130	-5.56 ± 3.66	99.98	97.46 ± 0.005
Method 2	Non-conjugated liposome (LP-DSPE-PEG-Maleimide)	121.63 ± 3.60	0.468 ± 0.075	-7.71 ± 0.175	-	98.80 ± 0.139
	Nisin-conjugated liposome (thiol-maleimide), LP:NS ratio 1:20	138.16 ± 3.81	0.043 ± 0.038	-1.43 ± 1.36	99.97	99.42 ± 0.154

- Size of nisin-conjugated liposome ranges from 130-230 nm, which is larger than non-conjugated liposome.
- Zeta potential, when nisin is conjugated with liposomes using method 1, shows a decrease in charge, while method 2 results in a more positive charge on surface.
- The ability of the conjugated liposomes to bind nisin is comparable, and their drug encapsulation efficiency is also similar.

Drug release analysis



- Drug release analysis showed that in Method 1, the non-conjugated liposomes had a faster release rate at 76.3% compared to the nisin-conjugated liposomes, which released 65.9%.
- In Method 2, the non-conjugated liposomes had a slower release rate at 59.3% compared to the nisin-conjugated liposomes, which released 99.2% over a period of 15 days.
- The Korsmeyer-Peppas model shows that non-conjugated LP-NH_2, nisin-conjugate (carbodiimide), and nisin-conjugate LP (thiol-maleimide) have n values in the range of $0.43 < n < 0.85$, indicating **anomalous diffusion**, which results from both drug diffusion and structural changes in the liposomes. In contrast, non-conjugated LP-maleimide has an n value of <0.43, indicating **fickian diffusion**, where drug diffusion occurs without structural changes in the liposomes.

Conclusion

- Conjugated liposome (method 2) are larger and have a more positive charge due to the conjugation with positively charged nisin.
- Thiol-maleimide bioconjugation (method 2) has more preferable physicochemical potential, with a size of 138.16 ± 3.81 nm and a charge of -1.43 ± 1.36 mV.
- Thiol-maleimide bioconjugation (method 2) has the fastest drug release rate, making it suitable for treatments that require rapid drug delivery.

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