Nisin-conjugated Liposomes Encapsulated with Doxorubicin as a Delivery System

Piyathida Wuthinonchai[1], **Panchika Prangkio**[1*]

[1]Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

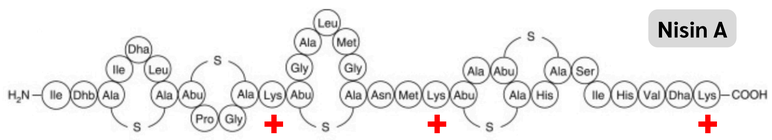
*Email: panchika.p@cmu.ac.th

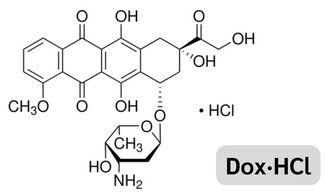
Abstract

Conventional drug delivery systems continue to face major limitations, including non-specific drug distribution, toxicity to normal tissues, and inadequate control of drug release, which necessitate the use of high drug doses and increase the risk of adverse side effects. This study aims to develop a doxorubicin (dox) delivery system based on nisin-conjugated liposomes using two bioconjugation strategies, carbodiimide and thiol–maleimide methods, when nisin was selected as a ligand due to its positive charges for targeting negatively charged membrane. The physicochemical properties of non-conjugated and nisin-conjugated liposomes were compared, and the release behavior and mechanisms of dox were investigated at pH 5.5 and 7.4, using appropriate kinetic models. Liposomes were prepared by the thin-film hydration method using soybean phosphatidylcholine and cholesterol as the main components, and dox was encapsulated via a remote loading technique. The results demonstrated that liposomes prepared by both methods exhibited an encapsulation efficiency of 99%. Nisin conjugation efficiencies obtained by the carbodiimide and thiol–maleimide methods were approximately 86% and 54%, respectively. Dynamic light scattering analysis revealed that the liposomes had an average particle size of approximately 120 nm with a narrow size distribution. Drug release studies were conducted using a dialysis method in a sodium phosphate buffer at pH 5.5 and 7.4 at 37°C for 8 days, and the release mechanisms were analyzed using the Korsmeyer–Peppas kinetic model. The results indicate that liposomes conjugated via the carbodiimide conjugation showed effective, pH-responsive drug release with high conjugation efficiency and >99% release in simulated at pH 5.5 while remaining stable at pH 7.4. These findings highlight the potential of nisin-conjugated liposomes as a controlled dox delivery system. Further studies at the cellular and animal levels would help to validate the efficacy and safety of the developed system and support its future medical application.

Introduction

- Cancer remains one of the leading cause of death, with ~14.8 M new cases and ~6.4 M deaths globally (2022).
- To overcome conventional drug delivery limitations, this research focuses on developing a **Targeted Drug Delivery System (TDDS)** using nanoparticle platforms to enhance therapeutic efficacy and reduce side effects.
- **Liposomes** were selected for their biocompatibility and dual-loading capacity for both hydrophilic (**Dox**) and lipophilic drugs. By functionalizing the surface with **Nisin** (a naturally derived cationic antimicrobial peptide), the resulting cationic charge promotes selective interaction with negatively charged cancer cell membranes within acidic microenvironments.

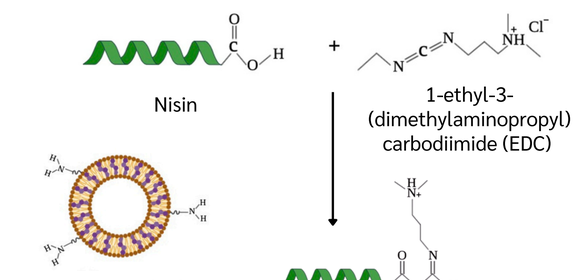




- This study aims to compare two bioconjugation strategies: **carbodiimide and thiol-maleimide conjugation.**

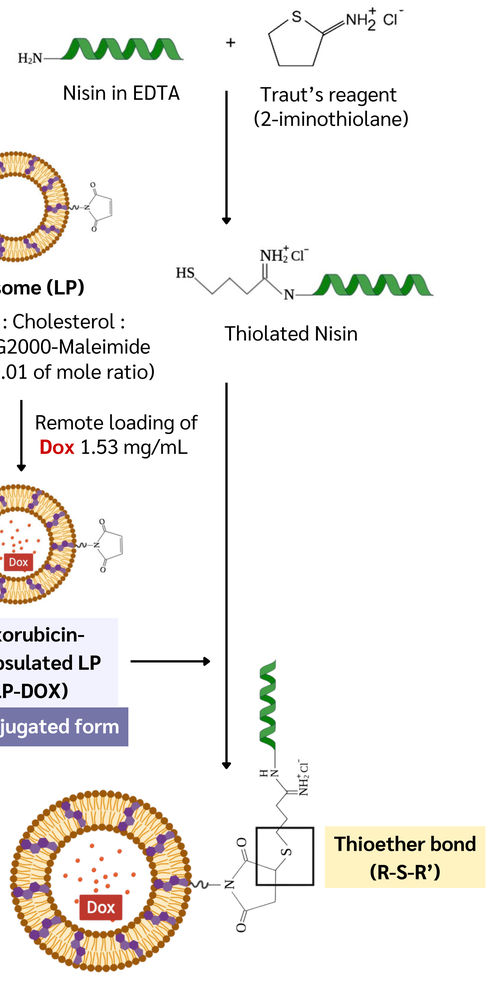
Conjugation method 1

Carbodiimide conjugation

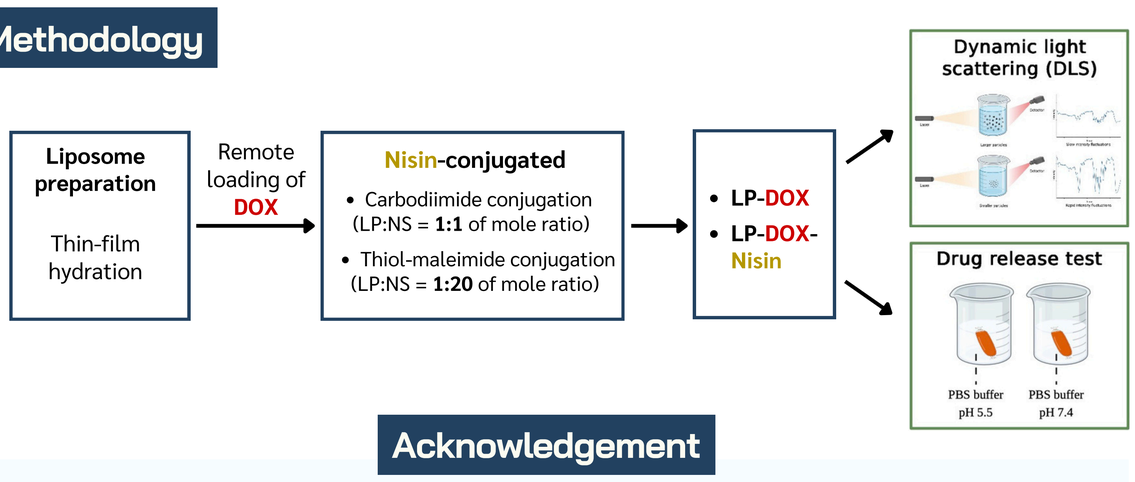


Conjugation method 2

Thiol-maleimide conjugation



Methodology



Results and Discussion

Characterization of Physicochemical Properties of Liposomes

Liposomes (LPs)		%Encapsulation efficiency	%Nisin conjugation	Size (nm)	Zeta potential (mV)	PDI
Carbodiimide conjugation	LP-DOX		-	120.4 ± 2.43	-21.8 ± 3.14	0.186
	LP-DOX-Nisin (LP:NS ratio 1:1)	99.16	86.16	121.3 ± 5.30	-18.1 ± 2.17	0.203
Thiol-maleimide conjugation	LP-DOX		-	115.5 ± 3.46	-25.6 ± 4.60	0.247
	LP-DOX-Nisin (LP:NS ratio 1:20)	99.02	53.72	116.8 ± 3.46	-22.1 ± 3.21	0.234

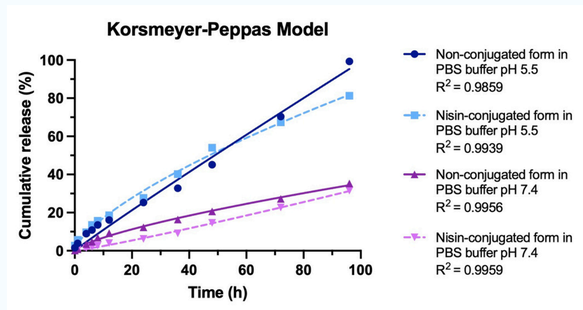
*Particle size and zeta potential of all liposomal formulations were measured by dynamic light scattering. The results are reported as mean ± SD (n=2)

- Both methods achieved an encapsulation efficiency of **~99%**.
- The Carbodiimide method achieved **higher** %nisin conjugation compared to the Thiol-maleimide method.
- Liposomes measured **~120 nm**, with nisin conjugation leading to slight increases in size and zeta potential due to electrostatic interactions. Furthermore, PDI values around **0.2** confirmed a homogeneous distribution, ensuring suitability for drug delivery.

Drug release analysis

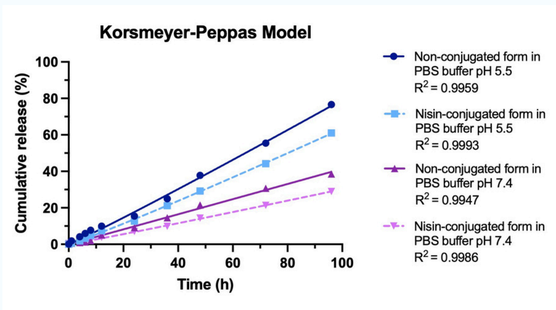
Method 1

Carbodiimide conjugation



Method 2

Thiol-maleimide conjugation



$$\frac{M_t}{M_\infty} = kt^n$$

- M_t / M_∞ : the proportion of the drug released at time 't'.
- k : a release rate constant of Korsmeyer
- n : the diffusion exponent characteristic of the release mechanism

Liposomes (LPs)			Diffusion Exponent (n)	Kinetic constant (K)
(Method 1) Carbodiimide conjugation	Drug release in pH 5.5 (Simulate tumor condition)	LP-DOX	0.957	1.210
		LP-DOX-Nisin (LP:NS ratio 1:1)	0.690	3.513
	Drug release in pH 7.4 (Simulate physiological condition)	LP-DOX	0.713	1.326
		LP-DOX-Nisin (LP:NS ratio 1:1)	1.121	0.188
(Method 2) Thiol-maleimide conjugation	Drug release in pH 5.5 (Simulate tumor condition)	LP-DOX	1.051	0.628
		LP-DOX-Nisin (LP:NS ratio 1:20)	1.071	0.458
	Drug release in pH 7.4 (Simulate physiological condition)	LP-DOX	1.007	0.401
		LP-DOX-Nisin (LP:NS ratio 1:20)	1.051	0.240

- The Korsmeyer-Peppas model indicates that for Method 1, the nisin-conjugated form at pH 5.5 and the non-conjugated form at pH 7.4 exhibit **Anomalous Transport** ($0.43 < n < 0.85$), where drug release is governed by both diffusion and structural relaxation of the lipid bilayer. Conversely, the non-conjugated form at pH 5.5 and the nisin-conjugated form at pH 7.4 follow **Super Case II transport** ($n > 0.85$), suggesting release mechanisms associated with structural erosion or liposomal transformation. However, all formulations in Method 2 show $n > 0.85$, classifying under **Super Case II transport.**
- Both conjugation methods exhibited significantly **higher drug release rates at pH 5.5** compared to pH 7.4. This pH-responsive behavior was most pronounced in Method 1
- Regarding the release constant (K), Method 1 showed that nisin conjugation effectively regulates drug release by **increasing K at pH 5.5** (targeted acceleration) while **decreasing** it **at pH 7.4** (leakage prevention under physiological conditions). In contrast, Method 2 **nisin conjugation leads to decreased K values** at both pH levels, suggesting nisin acts as a structural stabilizer or steric barrier that hinders overall release.

Conclusion

- Liposomes exhibited an average particle size of around 120 nm, with a polydispersity index (PDI) of approximately 0.2, indicating a sample homogeneity. However, the relatively low zeta potential suggests limited colloidal stability of the liposomes, which may increase the risk of particle aggregation.

	(Method 1) Carbodiimide conjugation	(Method 2) Thiol-maleimide conjugation
%Nisin conjugation	Higher (~86 %)	Lower (~54 %)
Stability	Intermediate	Intermediate
Release mechanism	Anomalous (non-fickian) transport and Super case II	Super case II
Drug release rate in pH 5.5	High >99%	High >75%
Drug release rate in pH 7.4	Slow	Slow and sustained

- **Carbodiimide-conjugated liposomes** showed effective drug release control. By providing high conjugation efficiency and an effective pH-responsive release profile, reaching >99% release at pH 5.5 while remaining stable at pH 7.4, this system demonstrates promising potential as a pH-responsive doxorubicin delivery system for cancer therapy.

Acknowledgement

- Drug Discovery Research Laboratory
- Materials Science Research Center, Faculty of Science
- Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University