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PREPARATION OF LIPOSOMES CONTAINING PROTEIN EXTRACT FROM
PASTEURELLA MULTOCIDA

FLIGHT LIEUTENANT SUKANYA ARPORNATTANAPONG

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สุกัญญา อารมณ์พัฒนาพงศ์ : การเตรียมไลโปโซมที่ใช้บรรจุสารสกัดโปรตีนจากเชื้อพาสเตอเรลลามัลโตซิดา (PREPARATION OF LIPOSOMES CONTAINING PROTEIN EXTRACT FROM PASTURELLA MULTOCIDA) อ.ที่ปรึกษา : รศ.ดร.อุบลทิพย์ นิมมานนิตย์, อ.ที่ปรึกษาร่วม : รศ.ดร.วิมลมาศ ลิปิพันธ์, 197 หน้า ISBN 974-633-334-8

ไลโปโซมที่ใช้บรรจุสารสกัดโปรตีนจากเชื้อพาสเตอเรลลามัลโตซิดา เตรียมโดยวิธีดัดเบิลอิมัลชันเทคนิค เพื่อศึกษาคุณสมบัติทางกายภาพ และความคงตัวของไลโปโซม เมื่อมีการเปลี่ยนแปลงของอัตราส่วนโดยน้ำหนักโมเลกุลของเลซิทีนจากไข่แดงต่อโคเลสเตอรอลที่ใช้เตรียมเป็นผนังของไลโปโซม อัตราส่วนโดยน้ำหนักโมเลกุลของเลซิทีนจากไข่แดงต่อโคเลสเตอรอลที่ถูกนำมาใช้เตรียมไลโปโซมคือ 1:0, 7:2 และ 1:1 จากการศึกษาพบว่าขนาดของอนุภาคและประสิทธิภาพในการเก็บกักสารของไลโปโซมขึ้นอยู่กับปริมาณโคเลสเตอรอลที่ใช้เตรียมไลโปโซม โดยไลโปโซมที่เตรียมจากเลซิทีนจากไข่แดงต่อโคเลสเตอรอลในอัตราส่วน 1:1 โดยน้ำหนักโมเลกุล ซึ่งเป็นไลโปโซมที่มีปริมาณโคเลสเตอรอลอยู่สูงนั้นจะมีขนาดอนุภาคใหญ่ (5.92 ไมครอน) และมีประสิทธิภาพในการเก็บกักสารสูง (45.26 ± 1.25 เปอร์เซ็นต์) และที่สำคัญคือมีความคงตัวสูง

ในการศึกษาครั้งนี้สารคาร์บอกซีเมทิลเซลลูโลสและคาร์บอกซีเมทิลไคโตแซน ซึ่งมีความเข้มข้น 0.02, 0.2 และ 0.5 เปอร์เซ็นต์โดยน้ำหนักต่อปริมาตรถูกนำมาใช้เพิ่มความคงตัวของไลโปโซม จากการศึกษาคุณสมบัติทางกายภาพ และความคงตัวของไลโปโซม พบว่าชั้นของโพลิเมอร์ที่หุ้มอยู่รอบอนุภาคไลโปโซมไม่ได้เป็นชั้นที่ต่อเนื่องกันแต่จะมีลักษณะเหมือนร่างแห และการใช้โพลิเมอร์เป็นตัวเพิ่มความคงตัวของไลโปโซมนั้นไม่มีผลรบกวนต่อโครงสร้างของเมมเบรน และประสิทธิภาพในการเก็บกักสาร การเพิ่มความเข้มข้นของโพลิเมอร์ทำให้ความคงตัวของไลโปโซม ในฟอสเฟตบัฟเฟอร์ที่มีค่าความเป็นกรดต่างเท่ากับ 7.4 ที่อุณหภูมิ 37 องศาเซลเซียสเพิ่มขึ้น การใช้คาร์บอกซีเมทิลไคโตแซน 0.5 เปอร์เซ็นต์โดยน้ำหนักต่อปริมาตรเป็นตัวเพิ่มความคงตัว พบว่าเปอร์เซ็นต์ของโปรตีนที่ถูกปลดปล่อยออกมาลดลงได้ถึง 60 เปอร์เซ็นต์ ภายในเวลา 10 ชั่วโมง คือจาก 99.05 เปอร์เซ็นต์ เหลือเพียง 33.02 เปอร์เซ็นต์ และไลโปโซมที่ถูกเคลือบด้วยโพลิเมอร์ทั้ง 2 ตัวนี้ สามารถคงปริมาณโปรตีนภายในอนุภาคไว้ได้มากกว่า 90 เปอร์เซ็นต์ เมื่อเก็บไว้ที่ 4 องศาเซลเซียสเป็นเวลา 3 เดือน

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ลายมือชื่อนิสิต
ลายมือชื่ออาจารย์ที่ปรึกษา
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Liposomes containing protein extract from *Pasteurella multocida* prepared by double emulsion technique were studied on the effect of molar ratio of egg yolk lecithin to cholesterol on their physicochemical properties and stability. The molar ratio of egg yolk lecithin to cholesterol used were 1:0, 7:2, and 1:1. The particle size and entrapping efficiency depended on the cholesterol content, 1:1 molar ratio of lecithin to cholesterol liposomes which was cholesterol rich liposomes gave the large particle sizes (5.92 μ m) and high entrapping efficiency (45.26 \pm 1.25%). Moreover, the higher cholesterol content gave a more stability of liposomes.

In this study, CM-Cellulose and CM-Chitosan in concentration of 0.02%, 0.2% and 0.5% w/v were used to stabilize liposomes. The physicochemical properties and stability of polymer coated liposomes were investigated. These polymers form mesh like layer around liposome vesicles. They do not disturb the structure of bilayer and entrapping efficiency. The more increasing concentrations of polymers, the stability of liposomes was increased in PBS pH 7.4 at 37°C. The percent of released protein from 0.5% w/v carboxymethylchitosan coated liposomes were reduced about 60% within 10 hours (from 99.05% to 33.02%). The polymer coated liposomes were kept in the closed seal containers, they could remain the protein content over 90% at 4°C for 3 months.

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LIST OF ABBREVIATIONS

°C	=	Degree Celsius
Chol	=	Cholesterol
CM-Cellulose	=	Carboxymethylcellulose
CM-Chitosan	=	Carboxymethylchitosan
g	=	Gravity
gm	=	Gram
hr	=	Hour
μ	=	Micron
μl	=	Microlitre
μg	=	Microgram
M	=	Molar
mg	=	Milligram
min	=	Minute
ml	=	Millilitre
mm	=	Millimetre
nm	=	Nanometre
PBS	=	Phosphate buffer solution
r^2	=	Correlation coefficient
SEM	=	Scanning electron microscope
SD	=	Standard deviation
TEM	=	Transmission electron microscope
UV	=	Ultraviolet