

การเตรียมผงพ่นแห้งของไลโซไซม์สำหรับนำส่งทางปอดโดยใช้ลิโปโซมเป็นตัวพา

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรคุษฎีบัณฑิต

สาขาวิชาเภสัชกรรม

คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2551

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

PREPARATION OF SPRAY-DRIED POWDERS OF LYSOZYME
FOR PULMONARY DELIVERY USING LIPOSOMES
AS A CARRIER

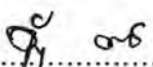
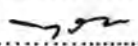
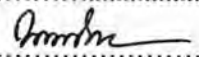
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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Pharmaceutics
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2008
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511649

คุณวุฒิ ขาววานิช : การเตรียมผงพ่นแห้งของไลโซไซม์สำหรับนำส่งทางปอดโดยใช้ลิโปโซม เป็นตัวพา (PREPARATION OF SPRAY-DRIED POWDERS OF LYSOZYME FOR PULMONARY DELIVERY USING LIPOSOMES AS A CARRIER) อ.ที่ปรึกษา วิทยานิพนธ์หลัก : รศ. ดร.พนั กุลวานิช, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ. ดร.นนทิมา วรธนะภูติ, 228 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อพัฒนา และหาสภาวะที่เหมาะสมของกระบวนการพ่นแห้งสำหรับเตรียมผงแห้งของลิโปโซมที่บรรจุไลโซไซม์ที่มีคุณสมบัติเหมาะสมสำหรับใช้นำส่งทางปอด ลิโปโซมที่ผ่านการลดขนาดเตรียมจากฟอสฟาติลโคลีนที่เกิดไฮโดรจีเนชัน (HPC) โดยมีและไม่มีคลอเลสเทอรอล จากนั้นผสมกับสารละลายของแมนิทอลและไลโซไซม์ก่อนนำไปพ่นแห้งได้ผงแห้งของลิโปโซม การวิจัยนี้ได้ศึกษาผลของอัตราส่วนระหว่าง HPC ต่อแมนิทอล การเติมไกลซินเพื่อเป็นสารช่วยลดการยึดติด และอัตราส่วนระหว่าง HPC ต่อคลอเลสเทอรอล ที่มีต่อคุณสมบัติของผงพ่นแห้งและลิโปโซมที่ได้จากการกระจายผงแห้งกลับคืน การประเมินผงพ่นแห้งที่เตรียมได้ตามฐานวิชา คุณสมบัติทางความร้อน และความเป็นผลึก โดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด เครื่องวัดปริมาณความร้อนแบบกราด และการเลี้ยวเบนรังสีเอ็กซ์ ตามลำดับ คุณสมบัติทางเคมีกายภาพของลิโปโซม ได้แก่ ประสิทธิภาพการกักเก็บ และการกระจายของขนาด ประเมินหลังจากการกระจายผงแห้งกลับคืนโดยใช้สภาวะที่สอดคล้องกับทางสรีรวิทยา แล้วนำสูตรตำรับที่เหมาะสมที่สุดไปศึกษาหาสภาวะการพ่นแห้งที่เหมาะสมต่อไป ศึกษาผลของปัจจัยทางกระบวนการพ่นแห้ง (อุณหภูมิเข้า อัตรารเร็วการป้อนสาร และปริมาณของแข็งทั้งหมด) ต่อคุณสมบัติของผงแห้ง (ปริมาณที่เตรียมได้ ปริมาณความชื้น ขนาดอนุภาค และประสิทธิภาพการกักเก็บ) โดยการออกแบบแฟคตอเรียลและการออกแบบส่วนประกอบกลาง ผลการศึกษาพบว่า กระบวนการพ่นแห้งสามารถใช้ในการเตรียมผงแห้งของลิโปโซมที่บรรจุไลโซไซม์ได้ กระบวนการพ่นแห้งไม่มีผลทำลายความคงตัวของฟอสโฟลิพิดทางโครงสร้างหลัก ระดับการเกาะกลุ่มขึ้นกับส่วนประกอบของผงแห้งอย่างมาก การเติมไกลซินทำให้เพิ่มปริมาณผงแห้งที่เตรียมได้ในส่วนรวบรวมของเครื่องพ่นแห้ง อย่างไรก็ตาม ไกลซินในปริมาณมากทำให้เกิดการเชื่อมติดกันของอนุภาคซึ่งป้องกันการเกิดลิโปโซมหลังจากการกระจายกลับคืน ที่อัตราส่วนระหว่างลิพิดต่อแมนิทอล 1:1 การเพิ่มปริมาณคลอเลสเทอรอลทำให้ขนาดของลิโปโซมที่ได้จากการกระจายกลับคืนลดลง และทำให้การกักเก็บของไลโซไซม์ในลิโปโซมเพิ่มขึ้น หลังจากกระจายผงแห้งกลับคืนใน Hepes Buffered Saline, pH 7.4 ที่ 37 °C ลิโปโซมสามารถเกิดขึ้นได้เองและกักเก็บไลโซไซม์เข้าไปในลิโปโซมได้อย่างมีประสิทธิภาพ กระบวนการพ่นแห้งไม่มีผลต่อฤทธิ์ทางชีวภาพของไลโซไซม์ แต่พบการเปลี่ยนแปลงทางโครงสร้าง จากการศึกษาการหาสภาวะที่เหมาะสมที่สุดพบว่าปัจจัยทางกระบวนการพ่นแห้งมีอิทธิพลที่มีนัยสำคัญต่อปริมาณผงแห้งที่เตรียมได้ ขนาดอนุภาค และประสิทธิภาพการกักเก็บ สภาวะการพ่นแห้งที่เหมาะสมที่เลือกจากการศึกษานี้สามารถเตรียมผงแห้งของลิโปโซมได้ปริมาณ 60.46 % ขนาดมัธยฐาน 6.00 ไมครอน และประสิทธิภาพการกักเก็บ 13.83 ไมโครกรัมไลโซไซม์ต่อมิลลิกรัมลิพิด

สาขาวิชา... เกษษกรรม.....ลายมือชื่อนิสิต  ๐๖
ปีการศึกษา...2551.....ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก 
ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม 

4676969433 : MAJOR PHARMACEUTICS

KEY WORD: LYSOZYME / LIPOSOMES / SPRAY DRYING / POWDERS / PULMONARY DELIVERY

DUSADEE CHARNVANICH : PREPARATION OF SPRAY-DRIED POWDERS OF LYSOZYME FOR PULMONARY DELIVERY USING LIPOSOMES AS A CARRIER. THESIS PRINCIPAL ADVISOR : ASSOC. PROF. POJ KULVANICH, Ph. D., THESIS COADVISOR : ASST. PROF. NONTIMA VARDHANABHUTI, Ph. D., 228 pp.

The purpose of this study was to develop and optimize the spray drying process for preparing spray-dried lysozyme-loaded liposomal powders with adequate properties for possible application in pulmonary delivery. Extruded liposomes were prepared from hydrogenated soybean phosphatidylcholine (HPC) with and without cholesterol (Chol). The extruded liposomes was then mixed with mannitol and lysozyme solutions and spray-dried into liposomal powders. The effects of HPC/mannitol ratio, presence of glycine as anti-adherent and HPC/Chol ratio on the properties of the spray-dried powders and the reconstituted liposomes were investigated. The spray-dried powders produced were characterized with respect to morphology, thermal property and crystallinity using scanning electron microscopy, differential scanning calorimetry and X-ray diffraction, respectively. Physicochemical properties of reconstituted liposomes, including encapsulation efficiency and size distribution, were evaluated after the powders were re-hydrated at physiologically relevant conditions. The most feasible formulation was further used for process optimization. The effects of spray drying process factors (inlet temperature, feed rate and total solid content) on the properties (yield, moisture content, particle size and entrapment efficiency) of the powders were investigated using the factorial design followed by the central composite design. The results indicated that the spray drying process was feasible in producing lysozyme-loaded liposomal powders. The process had no destructive effect on the stability of the major structural phospholipid. Degree of particle aggregation was strongly dependent on the composition of the powder. Though glycine could improve the yield in the collector of spray dryer, high amounts of glycine resulted in fusion of particles that prevented liposome formation upon reconstitution. At the lipid to mannitol ratio of 1:1, an increase in Chol resulted in a reduction of the size of the reconstituted liposomes. Entrapment of lysozyme in liposomes, on the other hand, increased with Chol content. After reconstitution with Hepes Buffered Saline, pH 7.4 at 37 °C, the powders spontaneously formed liposomes. Lysozyme was efficiently entrapped into the reconstituted liposomes. Biological activity of lysozyme was not affected by the spray drying process. However, some structural changes were evident. From the optimization study, the results indicated that the spray drying factors had a significant influence on the yield, the particle size and the entrapment efficiency of the powders. The optimum condition selected gave the liposomal powders with process yield of 60.46 %, mass median diameter of 6.00 μm and entrapment efficiency of 13.83 μg LSZ/mg lipid.

Field of study: ..Pharmaceutics... Student's signature

Academic year: 2008..... Principal advisor's signature

Co-advisor's signature

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ACKNOWLEDGEMENTS

I would like to express my special, sincere appreciation and gratitude to my thesis advisor, Associate Professor Dr. Poj Kulvanich, Department of Manufacturing Pharmacy and my thesis co-advisor, Assistant Professor Dr. Nontima Vardhanabhuti, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University for their invaluable advice, guidance, patience, kindness, encouragement and understanding throughout this study.

I am obliged to the members of thesis committee for their valuable scrutinizing and discussion.

I wish to express my thanks to Associate Professor Dr. Sunan Pongsamart, Department of Biochemistry for her supporting in Buchi spray dryer and Dr. Angkana Tantituvanont, Department of Pharmacy for her assistance and suggestion in experimental design.

A special appreciation is extend to Nattermann Phospholipid GmbH, Germany for kindly supplying Phospholipon® 90H.

Special thanks are extended to grants from the Graduate School of Chulalongkorn University, Faculty of Pharmaceutical Sciences and Strategic Scholarships Fellowships Frontier Research Networks of Commission on Higher Education.

Also, I would like to thank all the faculty members in the Department of Pharmacy for their assistance and encouragement.

Above all, I would like to express my sincere and deepest grateful to my family for their assistance, encouragement and understanding.

Finally, I gratefully acknowledge to all instructors, classmates and persons in Department of Pharmacy and Department of Manufacturing Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for their assistance.

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LIST OF ABBREVIATIONS

% CV	=	percent coefficient of variation
%w/w	=	percent weight by weight
%w/v	=	percent weight by volume
°C	=	degree Celsius
ANOVA	=	analysis of variance
C	=	total solid content (%w/w)
Chol	=	cholesterol
CI	=	confident interval
cm	=	centimeter
cm ³	=	cubic centimeter
Da	=	Dalton
df	=	degree of freedom
DPIs	=	dry powder inhalers
DRV	=	dehydrated-rehydrated vesicles
DSC	=	differential scanning calorimetry
D _{0.5} , MMD	=	mass median diameter
D _[4,3]	=	volume mean diameter
D _{aer}	=	theoretical aerodynamic diameter
Exp.	=	experiment
FT-Raman	=	Fourior Transform-Raman
g	=	gram
G	=	glycine
HBS	=	Hepes Buffered Saline (10 mM HEPES and 140 mM NaCl, pH 7.4)
HEPES	=	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)
HPC	=	hydrogenated soybean phosphatidylcholine
h	=	hour
kDa	=	kilodalton
kV	=	kilovolt

L	=	liter
LSZ	=	lysozyme
M	=	mannitol
MMAD	=	mass median aerodynamic diameter
MP	=	melting point
MPa	=	mega Pascal
MW	=	molecular weight
MWCO	=	molecular weight cutoff
mA	=	milli Amp
mg	=	milligram
min	=	minute
mL	=	milliliter
mM	=	millimolar
µg	=	microgram
µL	=	microlitre
µm, mcm	=	micrometer
µmol	=	micromole
NaCl	=	sodium chloride
nm	=	nanometer
no.	=	number
P	=	pump speed (%)
PC	=	polycarbonate
QPBCA	=	QuantiPro bicinchoninic acid
R ²	=	coefficient of determination
rpm	=	round per minute
RT	=	room temperature
SD	=	standard deviation
SDS-PAGE	=	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SE	=	standard error
sec	=	second
SEM	=	scanning electron microscope
SUV	=	small unilamellar vesicles

T	=	inlet temperature (°C)
TEM	=	transmission electron microscope
T_g	=	glass transition temperature
T_m, T_c	=	phase transition temperature
TLC	=	thin-layer Chromatography
v/v	=	volume by volume
W	=	watt
XRPD	=	x-ray powder diffraction