

การเตรียมและการตรวจสอบคุณสมบัติของเรตินิล ปาลมิเตต นีโอโซม



นางสาว นรธันันท์ ชวนสนิท

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเทคโนโลยีเภสัชกรรม หลักสูตรเทคโนโลยีเภสัชกรรม(นานาชาติ)

คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2544

ISBN 974-17-0727-4

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

**PREPARATION AND CHARACTERIZATION OF
RETINYL PALMITATE NIOSOMES**



Miss Nattanan Chuansanit

**A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmaceutical Technology
Pharmaceutical Technology (International) Program**

Faculty of Pharmaceutical Sciences

Chulalongkorn University

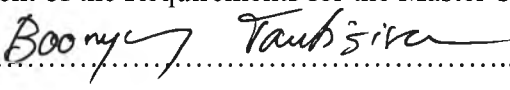
Academic Year 2001

ISBN 974-17-0727-4

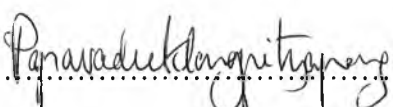
Copyright of Chulalongkorn University

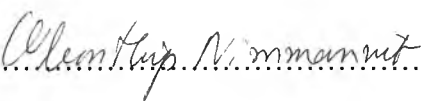
Thesis Title Preparation and Characterization of Retinyl Palmitate Niosomes
By Miss Nattanan Chuansanit
Field of study Pharmaceutical Technology (International)
Thesis Advisor Associate Professor Ubonthip Nimmannit, Ph.D.
Thesis Co-advisor Associate Professor Phensri Thongnopnua, Ph.D.


Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University
in Partial Fulfillment of the Requirements for the Master's Degree

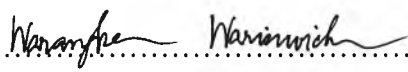
.....Dean of Faculty of
Pharmaceutical Sciences
(Associate Professor Boonyong Tantisira, Ph.D.)

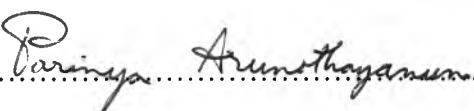
Thesis Committee

.....Chairman
(Associate Professor Papavadee Klongpityapong)

.....Thesis Advisor
(Associate Professor Ubonthip Nimmannit, Ph.D.)

.....Thesis Co-advisor
(Associate Professor Phensri Thongnopnua, Ph.D.)

.....Member
(Warangkana Warisnoicharoen, Ph.D.)

.....Member
(Parinya Arunothayanun, Ph.D.)

นรรจนันท์ ชวนสนิท:การเตรียมและการตรวจสอบคุณสมบัติของเรตินิล ปาลมิเทต นิโอโซม (PREPARATION AND CHARACTERIZATION OF RETINYL PALMITATE NIOSOMES)อ.ที่ปรึกษา : รศ. ดร.อุบลทิพย์ นิมมานันต์, อ.ที่ปรึกษาร่วม: รศ.ดร.เพ็ญศรี ทองนพเนื้อ ,173 หน้า. ISBN 974-17-0727-4

เรตินิล ปาลมิเทต นิโอโซมเตรียมโดยวิธีเขย่าด้วยมือ โดยใช้สารลดแรงตึงผิว สเปน 40, สเปน 60 หรือ สเปน 85 ร่วมกับ โคลเลสเตอร์อล และ โซลูแลน ซี 24 สร้างเป็นผนังของนิโอโซม โดยใช้อัตราส่วนต่อโมลของ สเปน:โคลเลสเตอร์อล:โซลูแลน ซี 24 เป็น 45:45:10 โดยมีปริมาณของส่วนที่เป็นไขมันและสารลดแรงตึงผิวทั้งหมด 300 ไมโครโมลใน 9 มิลลิลิตรหรือคิดเป็น 33.33 มิลลิโมลาร์ นิโอโซมที่ได้นี้มีรูปร่างกลมมีผนังชั้นเรียบเป็นชั้นหลายชั้น ขนาดโดยเฉลี่ยของนิโอโซมอยู่ในช่วง 10 ไมครอน เรตินิล ปาลมิเทต นิโอโซมได้ถูกเตรียมขึ้นเพื่อศึกษาหาความเหมาะสมในการกักเก็บยาในนิโอโซมโดยปรับปริมาณของ เรตินิล ปาลมิเทต เป็น 5,8 และ 10 มิลลิกรัม ตามลำดับ เปรอร์เซ็นต์การกักเก็บเรตินิลปาลมิเทตในนิโอโซมมีค่า 100 เปรอร์เซ็นต์เมื่อเติมเรตินิล ปาลมิเทต 5 มิลลิกรัมระหว่างการเตรียมนิโอโซม เมื่อศึกษาผลของโคลเลสเตอร์อล ต่อการกักเก็บยาโดยปรับเปลี่ยนอัตราส่วนโคลเลสเตอร์อล 0-80%โดยใช้ เรตินิลปาลมิเทต 8 มิลลิกรัม พบว่านิโอโซมที่เตรียมจากสเปน 40, สเปน 60 สามารถกักเก็บเรตินิล ปาลมิเทตได้น้อยลงเมื่อเพิ่มปริมาณโคลเลสเตอร์อลตรงกันข้ามกับนิโอโซมจากสเปน 85 ซึ่งสามารถกักเก็บเรตินิลปาลมิเทตได้มากขึ้น เมื่อปริมาณโคลเลสเตอร์อลสูงขึ้น ในการศึกษาการซึมผ่านของเรตินิล ปาลมิเทต นิโอโซมผ่านเมมเบรนของงูเห่า พบว่าเรตินิล ปาลมิเทตที่กักเก็บในนิโอโซมที่เตรียมจากสเปน 40 :โคลเลสเตอร์อล โซลูแลน ซี 24 (45:45:10) มีปริมาณการซึมผ่านเมมเบรนสะสมมากที่สุด และมีค่าอัตราการซึมผ่านสูงสุด.

ภาควิชา ลายมือชื่อนิสิต.....นรรจนันท์.....ชวสนิท.....
สาขาวิชา...เทคโนโลยีสารสนเทศ (นานาชาติ)...ลายมือชื่ออาจารย์ที่ปรึกษา.....
ปีการศึกษา.....2544.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4376855233 : MAJOR PHARMACEUTICAL TECHNOLOGY(INTERNATIONAL)PROGRAM

KEY WORD : RETINYL PALMITATE / NIOSOMES / ENTRAPMENT / NONIONIC SURFACTANT / SNAKE SKIN / IN VITRO PERMEATION.

NATTANAN CHUANSANIT. : PREPARATION AND CHARACTERIZATION OF RETINYL PALMITATE NIOSOMES. THESIS ADVISOR : ASSOC. PROF.UBONTHIP NIMMANNIT, Ph.D. THESIS CO-ADVISOR : ASSOC.PROF.PHENSRI THONGNOPNUA, Ph. D.

Retinyl palmitate niosomes were prepared by hand-shaking method using Span 40[®], Span 60[®] and Span 85[®], cholesterol and Solulan C-24[®] as the niosomal membranes. The molar ratio of Span:cholesterol:Solulan C-24[®] was 45:45:10 with the total lipid/surfactant of 300 micromoles in 9 ml (33.33 mM). The obtained niosomes were spherical and showed multilamellae with their mean diameter 10 microns. The retinyl palmitate loading optimization was studied with the concentration of retinyl palmitate varied from 5, 8 and 10 mg. The highest percentage of entrapment of retinyl palmitate was 100% when retinyl palmitate was added at 5 mg in niosome suspensions. The effect of cholesterol on retinyl palmitate entrapment was investigated with the molar ratio of cholesterol varying from 0-80% using 8 mg of retinyl palmitate. The higher the concentration of cholesterol was in niosomes, the lower the amount of retinyl palmitate could be entrapped in Span 40[®] and Span 60[®] niosomes. On contrary to Span 85[®], the entrapment of retinyl palmitate was increased with an increase of cholesterol prepared by Span 85[®]. The permeation of retinyl palmitate niosomes that were prepared by Span 40:cholesterol:Solulan C-24 (45:45:10) through the snake skin (Cobra) showed the highest cumulative retinyl palmitate and its flux.

Department -

Student's signature *Nattanan Chuansanit*

Field of study Pharmaceutical Technology (International) Advisor's signature *Ubonthip Nimmannit*

Academic year.....2001.....Co-advisor's signature *Phensri Thongnopnua*

ACKNOWLEDGEMENTS

I am deeply indebted to many people who have made their kind contributions to my study. First of all, I would like to express my profound gratitude and appreciation to my advisor, Associate Professor Dr. Ubonthip Nimmannit for her valuable comments and suggestions, her kindness and constant encouragement throughout my study.

My sincere appreciation also goes to Associate Professor Dr. Phensri Thongnopnua, my co-advisor for her helpful and valuable advice including her generous offer to use the circulating water bath apparatus for my experiments.

I am very much obliged to the members of my thesis committee for their scrutiny and discussion.

I would like to thank the Scientific and Technological Research Equipment Center of Chulalongkorn University for the supply of its useful data to my thesis.

Also, I am most grateful to the Pharmaceutical Technology (International) Program, Faculty of Pharmaceutical Sciences, Chulalongkorn University for granting partly financial support to conduct my investigation. I would also like to thank all the faculty members in the Pharmaceutical Technology (International) Program for their help and encouragement.

My special thanks are also extended to Mr. Jira Cheyklintes, the instructor of the Department of Vocational Education, Ministry of Education, for his kind assistance to modify the Franz diffusion cells ready for my permeation study.

Above all, I would like to express my sincerest and deepest gratitude to my parents for their love, understanding and support, particularly to Mom, for her endless love, care and encouragement throughout my life.

Finally, my deep appreciation goes to my friends and other people, whose names have not been mentioned, for helping me in anyway during the time of my study.

Thank you all.

CONTENTS

	PAGE
ABSTRACT (ENGLISH).....	iv
ABSTRACT (THAI).....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF TABLES	ix
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS.....	xxiii
CHAPTER	
I INTRODUCTION.....	1
II LITERATURE REVIEWS.....	7
III MATERIALS AND METHODS.....	39
IV RESULTS AND DISCUSSION.....	60
V CONCLUSIONS.....	105
REFERENCES.....	107
APPENDICES.....	114
APPENDIX I.....	115
APPENDIX II.....	120
APPENDIX III.....	125
APPENDIX IV.....	155
VITA.....	173

LIST OF TABLES

TABLE	PAGE
1. The types and features of niosomes.....	19
2. Accuracy data of retinyl palmitate niosome suspension (span 40: cholesterol: solulan C-24).....	62
3. Accuracy data of retinyl palmitate niosome suspension (span 60: cholesterol: solulan C-24).....	63
4. Accuracy data of retinyl palmitate niosome suspension (span 85: cholesterol: solulan C-24).....	63
5. Within run precision data.....	64
6. Between run precision data.....	65
7. The peak area ratio of standard solutions.....	68
8. The amount of entrapped and untrapped retinyl palmitate in niosome suspensions prepared by span 40: cholesterol: solulan C-24 with varying drug loading.....	76

TABLE	PAGE
9. The amount of entrapped and unentrapped retinyl palmitate in niosome suspensions prepared by span 60: cholesterol: solulan C-24 with varying drug loading.....	77
10. The amount of entrapped and unentrapped retinyl palmitate in niosome suspensions prepared by span 85: cholesterol: solulan C-24 with varying drug loading.....	78
11. The amount of entrapped and unentrapped retinyl palmitate in niosome suspensions prepared by span 40: cholesterol: solulan C-24 in different mole ratio.....	88
12. The amount of entrapped and unentrapped retinyl palmitate in niosome suspensions prepared by span 60: cholesterol: solulan C-24 in different mole ratio.....	89
13. The amount of entrapped and unentrapped retinyl palmitate in niosome suspensions prepared by span 85: cholesterol: solulan C-24 in different mole ratio.....	90

TABLE	PAGE
14. The cumulative amount of retinyl palmitate from niosomes prepared by span 40: cholesterol: solulan C-24(45:45:10).....	98
15. The cumulative amount of retinyl palmitate from niosomes prepared by span 60: cholesterol: solulan C-24 (45:45:10).....	99
16. The cumulative amount of retinyl palmitate from niosomes prepared by span 85: cholesterol: solulan C-24 (45:45:10).....	100
17. The data of particle size distribution for niosomes prepared by span 40:cholesterol:solulan C-24 (45:45:10).....	121
18. The data of particle size distribution for niosomes prepared by span 60:cholesterol:solulan C-24 (45:45:10).....	122
19. The data of particle size distribution for niosomes prepared by span 85:cholesterol:solulan C-24 (45:45:10).....	123
20. The statistical data of particle size of niosomes.....	124
21. The statistical data of the entrapped retinyl palmitate loading in niosomes prepared by span 40.....	134

TABLE	PAGE
22. The statistical data of the entrapped retinyl palmitate loading in niosomes prepared by span 60.....	135
23. The statistical data of the entrapped retinyl palmitate loading in niosomes prepared by span 85.....	136
24. The statistical data of the cholesterol effect on drug entrapment for span 40:cholesterol:solulan C-24 between (10:80:10) and (45:45:10).....	137
25. The statistical data of the cholesterol effect on drug entrapment for span 40:cholesterol:solulan C-24 between (10:80:10) and (65:25:10).....	138
26. The statistical data of the cholesterol effect on drug entrapment for span 40:cholesterol:solulan C-24 between (10:80:10) and (90:0:10).....	139
27. The statistical data of the cholesterol effect on drug entrapment for span 40:cholesterol:solulan C-24 between (45:45:10) and (65:25:10).....	140

TABLE	PAGE
28. The statistical data of the cholesterol effect on drug entrapment for span 40:cholesterol:solulan C-24 between (45:45:10) and (90:0:10).....	141
29. The statistical data of the cholesterol effect on drug entrapment for span 40:cholesterol:solulan C-24 between (65:25:10) and (90:0:10).....	142
30. The statistical data of the cholesterol effect on drug entrapment for span 60:cholesterol:solulan C-24 between (10:80:10) and (45:45:10).....	143
31. The statistical data of the cholesterol effect on drug entrapment for span 60:cholesterol:solulan C-24 between (10:80:10) and (65:25:10).....	144
32. The statistical data of the cholesterol effect on drug entrapment for span 60:cholesterol:solulan C-24 between (10:80:10) and (90:0:10).....	145

TABLE	PAGE
33. The statistical data of the cholesterol effect on drug entrapment for span 60:cholesterol:solulan C-24 between (45:45:10) and (65:25:10).....	146
34. The statistical data of the cholesterol effect on drug entrapment for span 60:cholesterol:solulan C-24 between (45:45:10) and (90:0:10).....	147
35. The statistical data of the cholesterol effect on drug entrapment for span 60:cholesterol:solulan C-24 between (65:25:10) and (90:0:10).....	148
36. The statistical data of the cholesterol effect on drug entrapment for span 85:cholesterol:solulan C-24 between (10:80:10) and (45:45:10).....	149
37. The statistical data of the cholesterol effect on drug entrapment for span 85:cholesterol:solulan C-24 between (10:80:10) and (65:25:10).....	150

TABLE	PAGE
38. The statistical data of the cholesterol effect on drug entrapment for span 85:cholesterol:solulan C-24 between (10:80:10) and (90:0:10).....	151
39. The statistical data of the cholesterol effect on drug entrapment for span 85:cholesterol:solulan C-24 between (45:45:10) and (65:25:10).....	152
40. The statistical data of the cholesterol effect on drug entrapment for span 85:cholesterol:solulan C-24 between (45:45:10) and (90:0:10).....	153
41. The statistical data of the cholesterol effect on drug entrapment for span 85:cholesterol:solulan C-24 between (65:25:10) and (90:0:10).....	154
42. The statistical data of RP flux from niosomes prepared by span 40 and span 60.....	168
43. The statistical data of RP flux from niosomes prepared by span 40 and span 85.....	169

TABLE	PAGE
44. The statistical data of RP flux from niosomes prepared by span 60 and span 85.....	170
45. The statistical data of cumulative RP from niosomes prepared by span 40 with different time intervals.....	171
46. The statistical data of cumulative RP from niosomes prepared by span 85 with different time intervals.....	172

LIST OF FIGURES

FIGURE	PAGE
1. Metabolism of vitamin A and its derivatives.....	3
2. Critical packing parameters for micelle, vesicle and reverse micelle formation.....	8
3. Physical chemistry of niosomes.....	10
4. Hydrophilic head groups found in vesicle forming amphiphiles.....	13
5. Hydrophobic tails found in vesicle forming amphiphiles.....	14
6. The structure of (a) cholesterol and (b) solulan C-24	15
7. The effect of the nature of the encapsulated drugs on the properties of the niosome dispersions.....	16
8. Hand-shaking method.....	20
9. Multilamellar vesicle formation.....	21
10. Multilamellar vesicle with entrapped drugs.....	21
11. The cross section of the skin structure and possible routes of penetration.....	25
12. The model of the stratum corneum and penetration pathway.....	31
13. The possible mechanisms involved in the effect of penetration enhancer.....	32
14. The UV spectrum of retinyl palmitate.....	60

FIGURE	PAGE
15. The UV spectrum of retinyl acetate.....	61
16. The chromatogram of retinyl palmitate in standard solution.....	66
17. The chromatogram of retinyl palmitate in the pellet.....	66
18. The chromatogram of unentrapped retinyl palmitate.....	67
19. The representative calibration curve of retinyl palmitate.....	68
20. Photographs of span 40:cholesterol:solulan C-24 niosomes	
span 60:cholesterol:solulan C-24 niosomes	
span 85:cholesterol:solulan C-24 niosomes	72
21. Scanning electron micrographs of retinyl palmitate niosomes	
prepared by (a) span 40 (b) span 60 (c) span 85.....	73
22. The percentage cumulative frequency undersize of retinyl palmitate	
niosomes prepared by span 40, span 60 and span 85.....	74
23. The entrapment of retinyl palmitate in niosomes prepared by	
span 40:cholesterol:solulan C-24 (45:45:10).....	79
24. The entrapment of retinyl palmitate in niosomes prepared by	
span 60: cholesterol: solulan C-24(45:45:10).....	80

FIGURE	PAGE
25. The entrapment of retinyl palmitate in niosomes prepared by span 85: cholesterol: solulan C-24(45:45:10).....	81
26. The entrapment efficiency of retinyl palmitate in 300 μ mole of total lipid/surfactant from span 40: cholesterol: solulan C-24 (45:45:10).....	82
27. The entrapment efficiency of retinyl palmitate in 300 μ mole of total lipid/surfactant from span 60: cholesterol: solulan C-24 (45:45:10).....	83
28. The entrapment efficiency of retinyl palmitate in 300 μ mole of total lipid/surfactant from span 85: cholesterol: solulan C-24 (45:45:10).....	84
29. The entrapment of retinyl palmitate in niosomes prepared by span 40 with different mole ratio of total lipid/surfactant.....	91
30. The entrapment of retinyl palmitate in niosomes prepared by span 60 with different mole ratio of total lipid/surfactant.....	92
31. The entrapment of retinyl palmitate in niosomes prepared by span 85 with different mole ratio of total lipid/surfactant.....	93

FIGURE	PAGE
32. Drug permeation profile of retinyl palmitate niosomes prepared by span 40:cholesterol:solulan C-24 (45:45:10), span 60:cholesterol:solulan C-24 (45:45:10) and span 85:cholesterol:solulan C-24 (45:45:10).....	101
33. The calibration curve of retinyl palmitate for within run precision (a) Sample No 1 (b) Sample No 2 (c) Sample No 3.....	126
34. a) The chromatogram of retinyl palmitate standard solution within run precision (Sample No 1).....	127
34. b) The chromatogram of retinyl palmitate standard solution within run precision (Sample No 2).....	128
34. c) The chromatogram of retinyl palmitate standard solution within run precision (Sample No 3).....	129
35. The calibration curve of retinyl palmitate for between run precision (a) Day 1 (b) Day 2 (c) Day 3.....	130
36. a) The chromatogram of retinyl palmitate standard solution between run precision (Day 1).....	131

FIGURE	PAGE
36. b) The chromatogram of retinyl palmitate standard solution between run precision (Day 2).....	132
36. c) The chromatogram of retinyl palmitate standard solution between run precision (Day 3).....	133
37. The calibration curve of retinyl palmitate permeation study.....	156
38. The chromatogram of retinyl palmitate standard solution with receptor fluid (a) RP 0.02 $\mu\text{g/ml}$ (b) RP 0.06 $\mu\text{g/ml}$	157
39. The chromatogram of retinyl palmitate standard solution with receptor fluid (a) RP 0.1 $\mu\text{g/ml}$ (b) RP 0.14 $\mu\text{g/ml}$	158
40. a) The chromatograms of permeable retinyl palmitate from niosomes prepared by span 40:cholesterol: solulan C-24 (45:45:10) at 3 Hr.....	159
40. b) The chromatograms of permeable retinyl palmitate from niosomes prepared by span 40:cholesterol: solulan C-24 (45:45:10) at 6 Hr.....	160
40. c) The chromatograms of permeable retinyl palmitate from niosomes prepared by span 40:cholesterol: solulan C-24 (45:45:10) at 9 Hr.....	161
41.a) The chromatograms of permeable retinyl palmitate from niosomes prepared by span 60:cholesterol: solulan C-24 (45:45:10) at 3 Hr.....	162

FIGURE	PAGE
41. b) The chromatograms of permeable retinyl palmitate from niosomes prepared by span 60:cholesterol: solulan C-24 (45:45:10) at 6 Hr.....	163
41. c) The chromatograms of permeable retinyl palmitate from niosomes prepared by span 60:cholesterol: solulan C-24 (45:45:10) at 9 Hr.....	164
42. a) The chromatograms of permeable retinyl palmitate from niosomes prepared by span 85:cholesterol: solulan C-24 (45:45:10) at 3 Hr.....	165
42. b) The chromatograms of permeable retinyl palmitate from niosomes prepared by span 85:cholesterol: solulan C-24 (45:45:10) at 6 Hr.....	166
42. c) The chromatograms of permeable retinyl palmitate from niosomes prepared by span 85:cholesterol: solulan C-24 (45:45:10) at 9 Hr.....	167

LIST OF ABBREVIATIONS

LUVs	=	large unilamellar vesicles
MLVs	=	multilamellar vesicles
mg	=	milligram
min	=	minutes
ml	=	millilitre
mM	=	millimole per litre
nm	=	nanometre
POE	=	polyoxyethylene
rpm	=	revolution per minute
RP	=	retinyl palmitate
R^2	=	coefficient of determination
SUVs	=	small unilamellar vesicles
SD	=	standard deviation
μm	=	micrometre
μg	=	microgram
μl	=	microlitre
μmole	=	micromole