

การพัฒนานาโนพาร์ติเคิลของไขมันแข็งและ  
ตัวพาไขมันโครงสร้างระดับนาโน บรรจุแอมโฟเทอริซิน บี

นาย ภาสวีร์ จันทร์สุก

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต

สาขาวิชาเภสัชอุตสาหกรรม ภาควิชาเภสัชอุตสาหกรรม

คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2548

ISBN 974-53-2486-8

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

DEVELOPMENT OF SOLID LIPID NANOPARTICLES AND  
NANOSTRUCTURED LIPID CARRIERS CONTAINING AMPHOTERICIN B

Mr. Phatsawee Jansook

A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in Pharmacy Program in Industrial Pharmacy

Department of Manufacturing Pharmacy

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2005

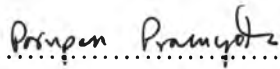
ISBN 974-53-2486-8

**481706**


Thesis Title            Development of solid lipid nanoparticles and nanostructured  
lipid carriers containing Amphotericin B  
By                            Mr. Phatsawee Jansook  
Field of Study            Industrial Pharmacy  
Thesis Advisor            Professor Garnpimol C. Ritthidej, Ph.D.

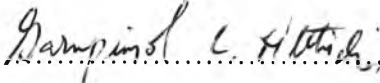
---

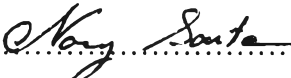
Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn  
University in Partial Fulfillment of the Requirements for the Master's Degree


.....Dean of the Faculty of  
Pharmaceutical Sciences  
(Associate Professor Pornpen Pramyothin, Ph.D.)

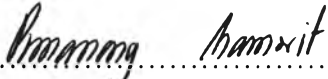
#### THESIS COMMITTEE

.....Chairman  
(Assistant Professor Wichein Thanindrataru, M.Sc. in Pharm.)

.....Thesis Advisor  
(Professor Garnpimol C. Ritthidej, Ph.D.)

.....Member  
(Professor Narong Sarisuta, Ph.D.)

.....Member  
(Narueporn Sutanthavibul, Ph.D.)

.....Member  
(Assistant Professor Pomanong Aramwit, Pharm. D., Ph.D.)

ภาควิธี จันทรสุก : การพัฒนานาโนพาร์ทิเคิลของไขมันแข็งและตัวพาไขมัน โครงสร้างระดับนาโนบรรจุแอมโฟเทอริซิน บี. (DEVELOPMENT OF SOLID LIPID NANOPARTICLES AND NANOSTRUCTURED LIPID CARRIERS CONTAINING AMPHOTERICIN B) อ. ที่ปรึกษา : ศ.ดร. กาญจน์พิมล ฤทธิเดช, 294 หน้า. ISBN 974-53-2486-8

แอมโฟเทอริซิน บี เป็นยาที่มีประสิทธิภาพต่อการต้านเชื้อราในกระแสเลือด แต่การใช้ฟิงจิโซนซึ่งเป็นยาในรูปแบบไมเซล ถูกจำกัดด้วยขนาดการใช้ที่ทำให้เกิดพิษต่อร่างกาย เพื่อลดความเป็นพิษของยาดังกล่าว ในการศึกษาครั้งนี้จึงได้พัฒนาระบบนำส่งยาในรูปแบบอนุภาคของไขมัน 2 ประเภท คือ นาโนพาร์ทิเคิลของไขมันแข็งและตัวพาไขมัน โครงสร้างระดับนาโน ซึ่งเตรียมขึ้นมาจาก กลีเซอรอลปีฮีนท และ กลีเซอรอลปาลมิโทส เตียเรทด้วยวิธี โฮโมจีไนเซชันอุณหภูมิสูงและวิธีไมโครอิมัลชัน เพื่อประเมินความคงตัวของอนุภาคทางกายภาพและความคงตัวทางเคมีของยาในสภาวะ 4 องศาเซลเซียส เป็นเวลา 3 เดือน ตัวพาไขมัน โครงสร้างระดับนาโนต่างจากนาโนพาร์ทิเคิลของไขมันแข็งเนื่องจากมีไขมันชนิดเหลวกระจายในเมทริกซ์ของไขมันแข็ง ผลการศึกษาพบว่า อนุภาคของไขมันที่เตรียมได้สามารถให้ทางหลอดเลือดดำ สูตรตำรับที่เหมาะสมมีขนาดอนุภาคน้อยกว่า 300 นาโนเมตร มีการกระจายขนาดอนุภาคช่วงแคบ และมีประจุลบที่ผิวของอนุภาค ด้วยสลายตัวเร็วในสูตรตำรับที่ใช้ ทวิน 80 และ ครีโมฟอร์ อีแอล เป็นสารเพิ่มความคงตัว สูตรตำรับที่ดีที่สุดในการเตรียมโดยวิธีโฮโมจีไนเซชันอุณหภูมิสูง ประกอบด้วย กลีเซอรอลปาลมิโทสเตียเรทร้อยละ 3 และ พอลลอกซามอร์ 407 ในความเข้มข้นร้อยละ 2 ส่วนในวิธีไมโครอิมัลชัน สูตรตำรับที่ดีที่สุดประกอบด้วย กลีเซอรอลปาลมิโทสเตียเรทร้อยละ 10 ครีโมฟอร์ อาร์เอช 40 และ กลีเซอรอลในความเข้มข้นร้อยละ 20 การเตรียมนาโนพาร์ทิเคิลของไขมันแข็งในรูปแบบผงแห้งไม่ประสบความสำเร็จ อาจเนื่องมาจากความไม่สมบูรณ์ของกระบวนการทำให้แห้ง ประสิทธิภาพของการบรรจุยาเพิ่มขึ้นอย่างมีนัยสำคัญเมื่อใช้ไขมันชนิดเหลวร่วมด้วยในระบบที่มี พอลลอกซามอร์ 407 เป็นสารเพิ่มความคงตัว โดยเฉพาะอย่างยิ่งถ้าใช้เลซิธินในระบบจะเพิ่มความสามารถในการบรรจุยามากขึ้น ผลของโปรตอนนิวเคลียร์แมกเนติกเรโซแนนซ์ ซึ่งให้เห็นว่ามีการรวมไขมันชนิดเหลวกระจายอยู่ในอนุภาคของแข็งหรืออยู่ระหว่างไขมันแข็งและชั้นของสารลดแรงตึงผิว สูตรตำรับที่เตรียมได้มีรูปแบบการจัดเรียงตัวของยาในลักษณะที่เป็นพิชต่อเซลล์และผลของการสลายตัวของเม็ดเลือดแดงต่ำกว่าฟิงจิโซน ซึ่งแสดงว่าระบบนำส่งดังกล่าวสามารถลดความเป็นพิษของยาได้ การปลดปล่อยยาจากระบบขึ้นกับการจัดเรียงตัวของยาและประสิทธิภาพในการบรรจุยา ในสูตรตำรับที่บรรจุยาร้อยละ 5 แสดงการปลดปล่อยยาเป็น 2 ช่วง การทดสอบฤทธิ์ต้านเชื้อโดยวิธีภายนอกร่างกายพบว่าทุกสูตรตำรับที่ได้รับการคัดเลือกมีประสิทธิภาพในการยับยั้งเชื้อได้เท่ากับหรือมากกว่าเมื่อเปรียบเทียบกับยาแอมโฟเทอริซิน บี และฟิงจิโซน จากการทดสอบโดยเทคนิคอินฟราเรดสเปกโทรสโกปี ไม่พบอันตรกิริยาระหว่างแอมโฟเทอริซิน บี และส่วนประกอบอื่นในตำรับ ผลของดีฟเฟอเรนเชียลสแกนนิ่งคาลอริเมทรี ฮอทสเตทไมโครสโกปี และเอกซเรย์ดิฟแฟรกโทเมทรี แสดงให้เห็นว่าแอมโฟเทอริซิน บี ที่อยู่ในเมทริกซ์ของไขมันกระจายอยู่ในระดับโมเลกุล หรือรูปอสัณฐาน ยกเว้นสูตรตำรับที่บรรจุยาร้อยละ 5

ภาควิชา.....เภสัชอุตสาหกรรม.....ลายมือชื่อนิสิต..... ภาควิธี จันทรสุก  
 สาขาวิชา.....เภสัชอุตสาหกรรม.....ลายมือชื่ออาจารย์ที่ปรึกษา.....  
 ปีการศึกษา.....2548.....

##4676611733 MAJOR : MANUFACTURING PHARMACY

KEYWORD : AMPHOTERICIN B / SOLID LIPID NANOPARTICLES /  
NANOSTRUCTURED LIPID CARRIERS / STABILITY

PHATSAWEE JANSOOK: DEVELOPMENT OF SOLID LIPID  
NANOPARTICLES AND NANOSTRUCTURED LIPID CARRIERS  
CONTAINING AMPHOTERICIN B. THESIS ADVISOR: PROF.  
GARNPIMOL C. RITTHIDEJ, Ph.D., 294 pp. ISBN 974-53-2486-8

Amphotericin B (AmB) is one of the most effective systemic antifungal agents, but its use is circumscribed by the dose-limiting toxicity of the conventional micellar dispersion formulation, Fungizone<sup>®</sup>. To lower its toxicity, AmB incorporated into aqueous dispersion of lipid nanoparticles was proposed. The paper describes the development of two types of lipid nanoparticles. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) made from glyceryl behenate (GB) and glyceryl palmitostearate (GP) were prepared by either hot high pressure homogenization (HPH) or warm microemulsion (WME) techniques in order to evaluate the physical stability of these particles, as well as the chemical stability of drug during storage at 4°C for 3 months. NLC differed from SLN by the presence of liquid lipid (glyceryl tricaprilate/caprinate) in the lipid matrix. The results indicated that these two lipid nanoparticles could be colloidal carriers for intravenous therapy. The optimized AmB-SLN formulations had an average diameter less than 300 nm, exhibited monodispersity and carried a negative charge. The AmB content was rapidly degraded when using tween 80 or cremophor EL as stabilizers. The best formulation of SLN dispersion prepared by HPH method consisted of 3% GP and 2% poloxamer 407 (P407), while the best formulation of SLN formulation prepared by WME method consisted of 10% GP, 20% cremophor RH40 and 20% glycerin. Lyophilized AmB-loaded SLN was not success possible due to incomplete lyophilization process. Entrapment efficiency of AmB was improved by blending small amounts of liquid oils with solid lipids. A significant increase in the encapsulation efficacy of NLC containing P407 was noted when compared to that of SLN formulation. In addition, the application of lecithin in the SLN dispersions could increase the amount of drug into the particles. The <sup>1</sup>H-NMR data indicated the formation of oil clusters within the solid nanoparticles or the presence between the solid platelet and the surfactant layer. Both AmB-SLN and AmB-NLC formulation have less aggregated species and hemolytic response than Fungizone<sup>®</sup> indicating that lipid nanoparticles could reduce its toxicity. The release profiles of AmB formulations depended on its aggregated form and entrapment efficiency. Furthermore, the formulation containing 5% AmB showed a drug release by a biphasic profile. For *in vitro* antifungal testing, all of the selected AmB formulations were equal and more effective than both AmB itself and Fungizone<sup>®</sup>. It was demonstrated by IR spectra that there was no chemical reaction occurred between AmB and other components. The DSC, HSM, X-ray diffractograms showed that AmB in lipid matrix was in either molecularly dispersed or amorphous form except the formulation containing 5% drug loading.

Department....Manufacturing Pharmacy.....Student's signature *Phatsawee Jansook*

Field of study...Industrial Pharmacy.....Advisor's signature *Garnpimol C. Ritthidej*

Academic year.....2005.....

## ACKNOWLEDGEMENTS

I would like to express my sincere thanks and gratitude to my thesis adviser, Professor Garnpimol C. Ritthidej, Ph.D. for her invaluable advice, guidance, encouragement and understanding. Her kindness and helpfulness are also deeply appreciated.

I also wish to express deep appreciation to Assistant Professor Wichein Thanindratarn, Professor Narong Sarisuta, Assistant Professor Pomanong Aramwit and Dr. Narueporn Sutanthavibul for spending their valuable times to be on my thesis committee and for their scrutinizing and discussion.

Special thanks are given to the Graduate School of Chulalongkorn University and the Ministry of University Affair for granting partial financial support to fulfill this study.

My sincere gratitude is extended to Associate Professor Rutt Sutthisri and Associate Professor Vimolmas Lipipun for proving  $^1\text{H-NMR}$  study and AmB susceptibility testing, respectively and also Miss Sombun Srimuang and Mrs. Tassanee Loenoo at Research Institute Unit, Ramathibadi Hospital for partial support in *in vitro* antifungal test

The other special thank to Research Institute Department, Government Pharmaceutical Organization, Thailand for supporting in particle size determination.

Also, I would like to express my infinite thanks and deepest gratitude to my friends, colleagues and staff members of the Department of Manufacturing Pharmacy for their assistance and encouragement.

Above all, I would like to express my thanks to my mother, my sisters and my brother for their assistance, care, cheerfulness, and encouragement.

Finally, I wish to thank other persons whose names have not been mentioned here for their assistance and encouragement.

# CONTENTS

	<b>Page</b>
THAI ABSTRACT.....	iv
ENGLISH ABSTRACT.....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF TABLES.....	ix
LIST OF FIGURES.....	xvi
ABBREVIATIONS.....	xxiii
CHAPTER	
I    INTRODUCTION.....	1
II   LITERATURE REVIEWS	
1. Solid lipid nanoparticles (SLN).....	5
2. Nanostructured lipid carriers (NLC).....	30
3. Amphotericin B (AmB).....	33
4. Lipid formulations of AmB.....	42
III  MATERIALS AND METHODS	
1. Formulation of SLN.....	56
2. Formulation of AmB loaded NLC.....	59
3. Physicochemical characterization of formulations.....	59
4. <i>In vitro</i> drug release.....	67
5. Biopharmaceutical characterizations of formulations.....	68
6. Stability studies.....	69
7. Statistical analysis.....	70
IV  RESULTS AND DISCUSSION	
1. Solid lipid nanoparticles (SLN).....	71
2. Nanostructured lipid carriers (NLC).....	98
3. Determination of drug content by HPLC method.....	101
4. Determination of entrapment efficiency (%EE).....	106
5. Morphology of AmB formulations.....	107
6. Physical and chemical stability studies.....	110

7. Effect of lecithin incorporated with AmB loaded SLN and NLC.....	127
8. Physical evaluations of various formulations.....	137
9. Model of four different AmB lipid particles.....	162
10. Effect of drug loading in various formulations.....	164
11. <i>In vitro</i> drug release.....	190
12. Biopharmaceutical characterizations of formulations.....	195
V CONCLUSIONS.....	202
REFERENCES.....	207
APPENDICES	
APPENDIX A.....	229
APPENDIX B.....	250
APPENDIX C.....	254
APPENDIX D.....	266
APPENDIX E.....	268
APPENDIX F.....	274
APPENDIX G.....	284
VITA.....	294



## LIST OF TABLES

<b>Table</b>	<b>Page</b>
1	Lipids used for preparation of solid lipid nanoparticles.....7
2	Stabilizers used for preparation of solid lipid nanoparticles.....8
3	Examples of drugs relevant for parenteral application incorporated into SLN.....14
4	Commercial available AmB formulations.....43
5	Chemical and physical properties of AmB formulations.....45
6	FDA-Approved indications for lipid formulations of AmB.....49
7	Adverse effects of AmB formulations.....50
8	AmB formulations commonly used in the treatment of invasive fungal infections .....52
9	Cost of treatment per day, at the recommended daily adult dose, for Fungizone <sup>®</sup> and the commercial lipid-based preparations.....52
10	Physical appearances of SLN dispersions containing 10% GB as solid lipid with different types and amounts of surfactants and co-surfactants prepared by WME method .....72
11	Physical appearances of SLN dispersions containing 10% GP as solid lipid with different types and amounts of surfactants and co-surfactants prepared by WME method.....72
12	The physical appearances of 3% GB-SLN and GP-SLN with 1-5% of Tw20, Tw80, CreEL and CreRH prepared by HPH method.....79
13	The physical appearances of 3% GB-SLN and GP-SLN with 1-5% of P188, P407, M52 and M59 prepared by HPH method.....81
14	The physical appearances of SLN containing AmB which solubilized by 0.1N NaOH and DMSO, 10% GP and 20% of various surfactant/co-surfactant prepared by WME method .....86
15	The particle sizes of SLN containing AmB which solubilized by 0.1N NaOH and DMSO, 10% GP and 20% of various surfactant/co-surfactant prepared by WME method.....87
16	The pH, osmolality and zeta potential of SLN containing AmB which solubilized by 0.1N NaOH and DMSO, 10% GP and 20% of various surfactant/co-surfactant prepared by WME method .....88

<b>Table</b>	<b>Page</b>
17 The physical appearances of 1% AmB loaded GB-SLN and GP-SLN dispersions with various stabilizers prepared by HPH method.....	90
18 Particle sizes of 1% AmB loaded GB-SLN and GP-SLN dispersions with various stabilizers prepared by HPH method, determined by LD and PCS....	91
19 The pH, osmolality and zeta potential of 1% AmB loaded GB-SLN and GP-SLN with various stabilizers prepared by HPH method.....	94
20 Effects of the nature of the bulking agents on the morphological characteristics of reconstituted AmB-SLN from freeze-dried products.....	95
21 The preparation yields of AmB freeze-dried products from GP-SLN dispersions containing 10%GP with 20% of various surfactants and co-surfactants prepared by WME method.....	96
22 The preparation yields of AmB freeze-dried products from GB-SLN and GP-SLN dispersions prepared by HPH method.....	96
23 Particle sizes of AmB freeze-dried products from GP-SLN dispersions prepared by WME method.....	97
24 Particle sizes of AmB freeze-dried products from GB-SLN and GP-SLN dispersions prepared by HPH method.....	98
25 The physical appearances of AmB loaded GB-NLC and GP-NLC with various stabilizers prepared by HPH method .....	99
26 Particle sizes of AmB loaded GB-NLC and GP-NLC with various stabilizers prepared by HPH method .....	100
27 The pH, osmolality and zeta potential of AmB loaded GB-NLC and GP-NLC with various stabilizers prepared by HPH method .....	100
28 Entrapment efficiency of AmB in various GP-SLN dispersions prepared by WME and HPH method.....	106
29 Entrapment efficiency of AmB in various GP-NLC dispersions prepared by HPH method.....	110
30 Particle sizes of AmB solubilized by 0.1N NaOH and DMSO loaded GP-SLN dispersions prepared by WME method at initial and after 3-months storage at 4°C.....	112
31 Particle sizes of AmB loaded GB-SLN and GP-SLN dispersions prepared by HPH method at initial and after 3-months storage at 4°C.....	113

<b>Table</b>	<b>Page</b>
32 The AmB content as a function of time at 4°C of GP-SLN dispersions prepared by WME method .....	116
33 The AmB content as a function of time at 4°C of GB-SLN and GP-SLN dispersions prepared by HPH method.....	118
34 Predicted shelf lives at 4°C of AmB in GP-SLN preparations prepared by WME method .....	119
35 Predicted shelf lives at 4°C of AmB in GB-SLN and GP-SLN preparations with various surfactants prepared by HPH method .....	120
36 The AmB content as a function of time at 4°C of lyophilized GP-SLN products prepared by WME method .....	121
37 The AmB content as a function of time at 4°C of lyophilized GB-SLN and GP-SLN products prepared by HPH method .....	123
38 Predicted shelf lives at 4°C of AmB in lyophilized GP-SLN preparations prepared by WME method .....	124
39 Predicted shelf lives at 4°C of AmB in lyophilized GB-SLN and GP-SLN preparations prepared by HPH method .....	124
40 Particle sizes of AmB loaded GB-NLC and GP-NLC with various surfactants prepared by HPH method at initial and after 3 months storage.....	125
41 The AmB content as a function of time at 4°C of GB-NLC and GP-NLC formulations with various surfactants prepared by HPH method.....	126
42 Predicted shelf lives at 4°C of AmB in GB-NLC and GP-NLC preparations prepared by HPH method.....	127
43 The physical appearances of AmB loaded SLN-L and NLC-L formulations prepared by HPH method.....	129
44 Particle sizes of AmB loaded SLN-L and NLC-L formulations with various stabilizers prepared by HPH method.....	130
45 The pH, osmolality and zeta potential of AmB loaded SLN-L and NLC-L formulations with various stabilizers prepared by HPH method.....	131
46 Entrapment efficiency of AmB in various SLN-L and NLC-L dispersions prepared by HPH method.....	132

<b>Table</b>	<b>Page</b>
47 Particle sizes of AmB loaded SLN-L and NLC-L formulations at initial and after 3 months storage.....	135
48 The AmB content as a function of time at 4°C of SLN-L and NLC-L formulations prepared by HPH method.....	136
49 Predicted shelf lives at 4°C of AmB in SLN-L and NLC-L formulations with various surfactants prepared by HPH method.....	137
50 Thermal behaviours of intact materials, drug-free and drug-loaded in various formulations stabilized by P407.....	145
51 The physical appearances of different amount of AmB loaded in various formulations.....	165
52 Particle sizes of different amount of AmB loaded in various formulations..	166
53 The pH of different amount of AmB loaded in various formulations.....	167
54 The osmolality of different amount of AmB loaded in various formulations.....	167
55 The zeta potential of different amount of AmB loaded in various formulations.....	168
56 Entrapment efficiency of different amount of AmB loaded in various formulations.....	169
57 Particle sizes of different amount of AmB loaded in various formulations at initial and 3 months storage .....	172
58 The AmB content as a function of time at 4°C of 2.5% and 5% AmB in various formulations.....	172
59 Predicted shelf lives at 4°C of different amount AmB loaded in various preparations.....	173
60 The thermal behaviours of different amount of AmB loaded in various formulations determined by DSC analysis.....	181
61 The thermal behaviours of 5% AmB loaded in various formulations and their physical mixture determined by DSC analysis.....	184
62 <i>In vitro</i> activities of various AmB formulations against important medical fungi.....	200

**Table****Page**

b1	Particle size of GP-SLN containing various type of surfactants and co-surfactants on the ratio of lipid: (surfactant:co-surfactant):water =10:(20:20):50.....	250
b2	Particle size of GP-SLN containing various type of surfactants and co-surfactants on the ratio of lipid: (surfactant:co-surfactant):water = 10:(25:25):50.....	250
b3	The equation and coefficients of determination of osmolality of GB-SLN dispersions prepared by WME method.....	251
b4	The equation and coefficients of determination of osmolality of GP-SLN dispersions prepared by WME method.....	251
b5	pH and osmolality of drug free SLN containing 3% GB or GP and various amount of Tw80 or Tw20.....	252
b6	pH and osmolality of drug free SLN containing 3% GB or GP and various amount of CreEL or CreRH.....	252
b7	pH and osmolality of drug free SLN containing 3% GB or GP and various amount of P118 or P407.....	253
b8	pH and osmolality of drug free SLN containing 3% GB or GP and various amount of M52 or M59.....	253
c1	Data of within run precision of AmB assayed by the HPLC method.....	258
c2	Data of between run precision of AmB assayed by the HPLC method.....	258
c3A	Data of accuracy of AmB assayed by the HPLC method (No. 1).....	259
c3B	Data of accuracy of AmB assayed by the HPLC method (No. 2).....	260
c3C	Data of accuracy of AmB assayed by the HPLC method (No. 3).....	260
c4A	Data of calibration curve of standard AmB solutions (No. 1).....	261
c4B	Data of calibration curve of standard AmB solutions (No. 2).....	262
c4C	Data of calibration curve of standard AmB solutions (No. 3).....	263
c5	Recovery of AmB from spiked placebo.....	265
d1	The characteristic bands of various AmB-WME spectra at the drug concentration range of 2.0 – 12.0 µg/ml.....	266
d2	The characteristic bands of various AmB-SLN spectra at the drug concentration range of 2.0 – 12.0 µg/ml.....	266

<b>Table</b>	<b>Page</b>
d3 The characteristic bands of various AmB-NLC spectra at the drug concentration range of 2.0 – 12.0 µg/ml.....	266
d4 The characteristic bands of various AmB-SLN-L spectra at the drug concentration range of 2.0 – 12.0 µg/ml.....	267
d5 The characteristic bands of various AmB-NLC-L spectra at the drug concentration range of 2.0 – 12.0 µg/ml.....	267
e1 Release of AmB from 1% AmB loaded SLN.....	269
e2 Release of AmB from 1% AmB loaded NLC.....	269
e3 Release of AmB from 1% AmB loaded SLN-L.....	270
e4 Release of AmB from 2.5% AmB loaded SLN.....	270
e5 Release of AmB from 2.5% AmB loaded NLC.....	271
e6 Release of AmB from 2.5% AmB loaded SLN-L.....	271
e7 Release of AmB from 5% AmB loaded SLN.....	272
e8 Release of AmB from 5% AmB loaded NLC.....	272
e9 Release of AmB from 5% AmB loaded SLN-L.....	273
g1 Hemolysis of sheep RBC at the varied levels of AmB as fungizone®.....	284
g2 Hemolysis of sheep RBC at the varied levels of AmB as AmB-WME3.....	284
g3 Hemolysis of sheep RBC at the varied levels of AmB as AmB-WME9.....	285
g4 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN1.....	285
g5 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN2.....	286
g6 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN3.....	286
g7 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN4.....	287
g8 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC1.....	287
g9 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC2.....	288
g10 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC3.....	288
g11 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC4.....	289
g12 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN-L1.....	289
g13 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN-L2.....	290
g14 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN-L3.....	290
g15 Hemolysis of sheep RBC at the varied levels of AmB as AmB-SLN-L4.....	291
g16 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC-L1.....	291
g17 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC-L2.....	292

**Table****Page**

g18 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC-L3....	292
g19 Hemolysis of sheep RBC at the varied levels of AmB as AmB-NLC-L4....	293

## LIST OF FIGURES

Figure	Page
1	Schematic procedure of hot and cold homogenization techniques for SLN production.....11
2	Models of incorporation of active compounds into SLN.....15
3	The three types of NLC compared to the relatively ordered matrix of SLN .....32
4	The chemical structure of AmB.....34
5	The particle sizes of drug-free SLN dispersions containing 10%GP as solid lipid with 20% and 25% of surfactant/co-surfactant prepared by WME method .....75
6	The pH of drug-free SLN dispersions with various types and amount of surfactants/co-surfactants prepared by WME.....76
7	The osmolality of drug-free SLN with various types and amount of surfactants/co-surfactants prepared by WME.....77
8	The particle sizes of drug-free SLN dispersions containing 3% GB and GP as solid lipid with 2% of various surfactants prepared by HPH method .....82
9	The pH of drug-free SLN dispersions with various types and amount of surfactants prepared by HPH.....83
10	The osmolality of drug-free SLN with various types and amount of surfactants prepared by HPH.....84
11	The particle size distribution determined by LD of 1% AmB loaded GB-SLN (I) and GP-SLN (II) with various surfactants prepared by HPH method .....92
12	The percentage of AmB content using 0.1N NaOH and DMSO as solubilizing agent in various GP-SLN formulations prepared by WME method.....102
13	The percentage of AmB content in various GB-SLN and GP-SLN formulations prepared by HPH method.....102
14	Comparison of the AmB content solubilized by 0.1N NaOH and DMSO in various GP-SLN dispersions to lyophilized form prepared by WME method....104



<b>Figure</b>	<b>Page</b>
15 Comparison of the AmB content in various GB-SLN and GP-SLN dispersions to lyophilized form prepared by HPH method.....	104
16 The percentage of AmB content in various GB-NLC and GP-NLC formulations prepared by HPH method .....	105
17 The TEM micrographs of AmB loaded GP-SLN dispersions prepared by WME method.....	108
18 The TEM micrographs of AmB loaded GP-SLN dispersions prepared by HPH method.....	109
19 The Cryo-SEM micrographs of AmB loaded GP-SLN dispersion with 2% P407 and GB-SLN dispersion with 2% P407.....	109
20 The TEM micrographs of AmB loaded GP-NLC dispersions prepared by HPH method.....	111
21 The Cryo-SEM micrographs of AmB loaded GP-NLC dispersion with 2% P407 and GB-NLC dispersion with 2% P407.....	111
22 The percentage of AmB content in GP-SLN dispersions containing CreRH with various co-surfactants prepared by WME method after 3 months storage at 4°C and 30°C.....	114
23 The percentage of AmB content in GP-SLN dispersions with various surfactants prepared by HPH method after 3 months storage at 4°C and 30°C.....	114
24 AmB residual content in various GP-SLN formulations prepared by WME method at 4°C as a function of time (AmB solubilized in 0.1N NaOH) .....	117
25 AmB residual content in various GP-SLN formulations prepared by WME method at 4°C as a function of time (AmB solubilized in DMSO) .....	117
26 AmB residual content in various GB-SLN formulations prepared by HPH method at 4°C as a function of time.....	118
27 AmB residual content in various GP-SLN formulations prepared by HPH method at 4°C as a function of time.....	119
28 AmB residual content at 4°C in various lyophilized GP-SLN formulations as a function of time (AmB solubilized by 0.1N NaOH, prepared by WME method).....	122

<b>Figure</b>	<b>Page</b>
29 AmB residual content at 4°C in various lyophilized GP-SLN formulations as a function of time (AmB solubilized by DMSO, prepared by WME method).....	122
30 AmB residual content at 4°C in various lyophilized GB-SLN and GP-SLN formulations as a function of time (prepared by HPH method).....	123
31 AmB residual content at 4°C in various GB-NLC and GP-NLC formulations prepared by HPH method as a function of time .....	127
32 The percentage of AmB content in various SLN-L and NLC-L formulations prepared by HPH method.....	131
33 The TEM micrographs of AmB-SLN-L and AmB-NLC-L formulations stabilized by P407 prepared by HPH method.....	133
34 The Cryo-SEM micrographs of AmB-SLN-L and AmB-NLC-L formulations stabilized by P407 prepared by HPH method.....	134
35 AmB residual content at 4°C in various SLN-L and NLC-L formulations as a function of time.....	136
36 IR spectra of AmB(A); GP(B); CreRH (C);and lipid pellets of preparations AmB-WME1 (D); AmB-WME2 (E); AmB-WME3 (F).....	140
37 IR spectra of AmB(A); GP (B); P407(C); MCT oil(D) and lipid pellets of preparations AmB-SLN (E); AmB-NLC (F) stabilized by P407.....	141
38 IR spectra of AmB(A); GP(B); P407(C); MCT oil(D); PL(E) and lipid pellets of preparations AmB-SLN-L (F); AmB-NLC-L (G) stabilized by P407.....	142
39 DSC thermograms of GP (A); GB (B); P407 (C); PL (D); and AmB (E)....	146
40 DSC thermograms of lipid matrices of preparations containing drug-free GB-SLN (A); 1%AmB loaded GB-SLN (B); drug-free GB-NLC (C); and 1%AmB loaded GB-NLC (D).....	146
41 DSC thermograms of lipid matrices of preparations containing drug-free GP-SLN (A); 1%AmB loaded GP-SLN (B); drug-free GP-NLC (C); and 1%AmB loaded GP-NLC (D).....	147

<b>Figure</b>	<b>Page</b>
42 DSC thermograms of lipid matrices of preparations containing drug-free GP-SLN-L (A); 1%AmB loaded GP-SLN-L (B); drug-free GP-NLC-L (C); and 1%AmB loaded GP-NLC-L (D).....	147
43 HSM microphotographs of (I)unmelt GB; (II)melting GB to (III)totally melt GB (magnification 40x) obtained from lyophilized 1%AmB-SLN (A), 1%AmB-NLC (B).....	149
44 HSM microphotographs of (I)unmelt GP; (II)melting GP to (III)totally melt GP (magnification 40x) obtained from lyophilized 1%AmB-SLN3 (A), 1%AmB-NLC3 (B), 1%AmB-SLN3-L (C) and 1%AmB-NLC3-L (D).....	149
45 (a)X-ray diffractogram of AmB(A); P407(B); PL(C) and GP(D); (b)1%AmB-SLN(A); 1%AmB-NLC(B); 1%AmB-SLN-L(C) and 1%AmB-NLC-L (D).....	152
46 The 500 MHz <sup>1</sup> H-NMR spectrum of GP in CDCL <sub>3</sub> (A); P407 in D <sub>2</sub> O(B); MCT oil in CDCL <sub>3</sub> (C); AmB-SLN in D <sub>2</sub> O(D); AmB-NLC in D <sub>2</sub> O(E).....	155
47 The 500 MHz <sup>1</sup> H-NMR spectrum of PL in D <sub>2</sub> O(a); AmB-SLN-L in D <sub>2</sub> O(b); AmB-NLC-L in D <sub>2</sub> O(c).....	156
48 UV-Visible absorption spectra of AmB solubilized in DMSO:MeOH (1:999 %v/v) (A);AmB in PBS, pH 7.4 (B) and Fungizone <sup>®</sup> in PBS, pH 7.4 (C); (b): various AmB-SLN prepared by WME method. AmB-WME1 (A), AmB-WME2 (B) and AmB-WME3 (C); (c,d,e,f): various AmB-SLN, AmB-NLC, AmB-SLN-L, AmB-NLC-L formulation in PBS, pH 7.4 prepared by HPH method, stabilized by Tw20(A), CreRH(B), P407(C), M52(D).....	158
49 The absorbances peakI/peakIV ratio of AmB in various formulations as function of concentrations; (a): AmB in DMSO:MeOH (1:999%v/v); AmB and Fungizone in isotonic PBS, pH 7.4; (b): AmB-SLN formulations prepared by WME method; (c, d, e, f): AmB-SLN, AmB-NLC, AmB-SLN-L and AmB-NLC-L formulations prepared by HPH method.....	159
50 Models of AmB loaded different lipid nanoparticles.....	163

<b>Figure</b>	<b>Page</b>
51 Shear stress vs. shear rate plots for various amount AmB loaded NLC, SLN-L and NLC-L formulations.....	165
52 The percentage of AmB content of different amount loaded in various formulations. ....	168
53 The Cryo-SEM micrographs of AmB-SLN (top), AmB-NLC (middle), AmB-SLN-L (bottom); 2.5, 5% AmB loaded (left, right).....	170
54 AmB residual contents at 4°C of 2.5% and 5% loaded in various formulations as a function of time.....	173
55 IR spectra of AmB-SLN containing 1% AmB(A); 2.5%AmB(B); 5%AmB(C) and physical mixture of lyophilized SLN base and 5% of AmB powder(D).....	175
56 IR spectra of AmB-NLC containing 1% AmB(A); 2.5%AmB(B); 5%AmB(C) and physical mixture of lyophilized NLC base and 5% of AmB powder(D).....	176
57 IR spectra of AmB-SLN-L containing 1% AmB(A); 2.5%AmB(B); 5%AmB(C) and physical mixture of lyophilized SLN-L base and 5% of AmB powder(D).....	177
58 IR spectra of AmB-NLC-L containing 1% AmB(A); 2.5%AmB(B); 5%AmB(C) and physical mixture of lyophilized NLC-L base and 5% of AmB powder(D).....	178
59 DSC thermograms of lipid matrices of preparations containing 1, 2.5, 5%AmB-SLN(A,B,C); 1, 2.5, 5%AmB-NLC(D,E,F); 1, 2.5, 5% AmB-SLN-L(G,H,I); 1, 2.5, 5%AmB-NLC-L(J,K,L).....	180
60 DSC thermograms of 5% AmB lipid matrix and physical mixture of of SLN (A,B); NLC(C,D); SLN-L(E,F) and NLC-L(G,H), respectively .....	183
61 The comparison between the degree of crystallinity of 5% AmB physical mixture and 5% AmB loaded SLN, SLN-L, NLC and NLC-L formulations.....	184
62 The depression of melting temperature of the various lipid particles formulations; each point was average from three drug loading data.....	185

<b>Figure</b>	<b>Page</b>
63 HSM microphotographs of (I)unmelt GP, (II) melting GP to (III) totally melt GP (magnification 40x) obtained from lyophilized 1, 2.5, 5% (top, middle, bottom) of AmB loaded SLN(A); NLC(B); SLN-L(C) and NLC-L(D).....	187
64 X-ray diffractogram of (i)PM of GP+AmB(A), GP+P407+AmB(B) and GP+P407+PL+AmB(C); (ii)PM of 5% AmB and the lyophilized base of SLN(A), NLC(B), SLN-L(C) and NLC-L(D); (iii)1, 2.5, 5% (A,B,C) of lyophilized AmB-SLN; (iv) 1, 2.5, 5% (A,B,C) of lyophilized AmB-NLC;(v)1, 2.5, 5% (A,B,C) of lyophilized AmB-SLN-L; (vi) 1, 2.5, 5% (A,B,C) of lyophilized AmB-NLC-L.....	189
65 The release profile of AmB in SLN, NLC and SLN-L formulations 1.0%(a), 2.5%(b), 5%(c).....	192
66 The release profile of formulations containing 1.0%, 2.5% and 5.0% AmB loaded SLN (a); NLC (b); SLN-L (c).....	194
67 Hemolysis of sheep RBC at the varied levels of AmB in formulations; (a): Fungizone; (b): AmB-SLN prepared by WME method; (c), (d), (e), (f): AmB-SLN, AmB-NLC, AmB-SLN-L and AmB-NLC-L prepared by HPH method.....	196
c1 The UV spectrum of AmB dissolved in DMSO: MeOH (1:999 %v/v).....	254
c2 The HPLC chromatograms of water (A); PBS pH 7.4 (B); DMF (C); DMSO (D); and the extraction of drug-free prepared by WME method (WME1 (E); WME2 (F); WME3 (G)).....	255
c3 The HPLC chromatograms of the extraction of drug-free prepared by HPH method; SLN1 (A), SLN2 (B), SLN3 (C), SLN4(D), NLC1 (E), NLC2 (F), NLC3 (G), NLC4 (H), SLN-L (I) and NLC-L (J).....	256
c4 The HPLC chromatograms of the standard solutions of AmB; 0.8 µg/ml (A); 1.2 µg/ml (B); 1.6 µg/ml (C); 2.0 µg/ml (D); 3.0 µg/ml (E); 4.0 µg/ml (F); 6.0 µg/ml (G); and 8.0 µg/ml (H).....	257
c5A Calibration curve of AmB assay by HPLC method (No.1).....	261
c5B Calibration curve of AmB assay by HPLC method (No.2).....	262
c5C Calibration curve of AmB assay by HPLC method (No.3).....	263

<b>Figure</b>	<b>Page</b>
c6 The linear regression analysis between the average amount recovered and the average amount added.....	265
e1 The calibration curve of AmB dissolved in PBS, pH 7.4.....	268
f1A The 500 MHz <sup>1</sup> H-NMR spectrum of GP in CDCl <sub>3</sub> .....	274
f1B The 500 MHz <sup>1</sup> H-NMR spectrum of GP in CDCl <sub>3</sub> .....	274
f1C The 500 MHz <sup>1</sup> H-NMR spectrum of GP in CDCl <sub>3</sub> .....	275
f2A The 500 MHz <sup>1</sup> H-NMR spectrum of MCT oil in CDCl <sub>3</sub> .....	275
f2B The 500 MHz <sup>1</sup> H-NMR spectrum of MCT oil in CDCl <sub>3</sub> .....	276
f3A The 500 MHz <sup>1</sup> H-NMR spectrum of P407 in D <sub>2</sub> O.....	276
f3B The 500 MHz <sup>1</sup> H-NMR spectrum of P407 in D <sub>2</sub> O.....	277
f4A The 500 MHz <sup>1</sup> H-NMR spectrum of PL in CDCl <sub>3</sub> .....	277
f4B The 500 MHz <sup>1</sup> H-NMR spectrum of PL in CDCl <sub>3</sub> .....	278
f4C The 500 MHz <sup>1</sup> H-NMR spectrum of PL in CDCl <sub>3</sub> .....	278
f5A The 500 MHz <sup>1</sup> H-NMR spectrum of AmB-SLN in D <sub>2</sub> O.....	279
f5B The 500 MHz <sup>1</sup> H-NMR spectrum of AmB-SLN in D <sub>2</sub> O.....	279
f6A The 500 MHz <sup>1</sup> H-NMR spectrum of AmB-NLC in D <sub>2</sub> O.....	280
f6B The 500 MHz <sup>1</sup> H-NMR spectrum of AmB-NLC in D <sub>2</sub> O.....	280
f7A The 500 MHz <sup>1</sup> H-NMR spectrum of AmB-SLN-L in D <sub>2</sub> O.....	281
f7B The 500 MHz <sup>1</sup> H-NMR spectrum of AmB-SLN-L in D <sub>2</sub> O.....	281
f7C The 500 MHz <sup>1</sup> H-NMR spectrum of AmB-SLN-L in D <sub>2</sub> O.....	282
f8A The 500 MHz <sup>1</sup> H-NMR spectrum of AmB-NLC-L in D <sub>2</sub> O.....	282
f8B The 500 MHz <sup>1</sup> H-NMR spectrum of AmB-NLC-L in D <sub>2</sub> O.....	283

**ABBREVIATIONS**

%	percentage
°C	degree Celsius (centigrade)
µg	microgram (s)
µl	microlitre (s)
µm	micrometer (s)
$\lambda_{\max}$	wavelength of maximum absorption
<	less than
>	more than
<sup>1</sup> H-NMR	proton nuclear magnetic resonance spectroscopy
ABCD <sup>+</sup>	AmB colloidal dispersion
AFM	atomic force microscopy
AmB	amphotericin B
AmBD	AmB deoxycholate
ABL <sub>C</sub>	AmB lipid complex
AUC	area under the curve
AWP	average wholesale price
BCG	Bacillus of Calmette and Guérin
cm <sup>-1</sup>	the reciprocal of centimeter
C <sub>max</sub>	maximum plasma concentration
CFT	critical flocculation temperature
CMC	critical micelle concentration
CreEL	cremophor EL
CreRH	cremophor RH40
Cryo-SEM	cryo-scanning electron microscopy
CSF	cerebrospinal fluid
CV	coefficient of variation
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DSC	differential scanning calorimetry
e.g.	for example, <i>exempli gratia</i>
EE	entrapment efficiency

ESR	electron spin resonance
et.al.	et alli, and others
FD	freeze-dried
FDA	Food and Drug Administration
FFF	field-flow fractionation
FT-IR	fourier transform infrared
g	gram (s)
GB	glyceryl behenate
Gly	glycerin
GP	glyceryl palmitostearate
GRAS	Generally recognized as safe (US food and drug)
HLB	hydrophilic lipophilic balance
HPH	high pressure homogenization
HPLC	high-performance liquid chromatography
h	hour (s)
HSM	hot stage microscopy
i.e.	id est (that is)
i.m.	intramuscular
i.v.	intravenous
IR	infrared
km	kilometer (s)
LAF	laminar air flow cabinet
L-AmB	Liposomal AmB
LD	laser diffraction
LDL	low-density lipoprotein
LDH	lactate dehydrogenase
log	logarithm
M52	myrj 52
M59	myrj 59
MCT	medium chain triglyceride
MeOH	methanol
MFC	minimum fungicidal concentration
mg	milligram (s)
MIC	minimum inhibitory concentration



min	minute (s)
ml	milliliter (s)
mOsm/L	milliosmols per liter
MPS	mononuclear phagocytic system
MRT	mean residence times
mV	millivolt (s)
MW	molecular weight
N	normality
NCCLS	National Committee for Clinical Laboratory Standards
NLC	nanostructured lipid carriers
nm	nanometer (s)
NMR	magnetic resonance spectroscopy
No.	number of sample
OTC	oxytetracycline
o/w	oil in water emulsion
P118	poloxamer 118
P407	poloxamer 407
PCS	photon correlation spectroscopy
PEG	polyethylene glycol 400
PG	propylene glycol
pH	the negative logarithm of the hydrogen ion concentration
PI	polydispersity index
PL	phospholipon <sup>®</sup> 90H
PM	physical mixture
po	peroral
ppm	parts per million
psi	pound (s) per square inch
PXRD	powder X-ray diffractometry
R <sup>2</sup>	coefficient of determination
RBCs	red blood cells
RES	reticuloendothelial system
rpm	revolution (s) per minute

RT	retention time
SAXS	small angle X-ray scattering
s.c.	subcutaneous
SD	standard deviation
SLM	solid lipid microparticles
SLN	solid lipid nanoparticles
TEM	transmission electron microscopy
TGF	tubuloglomerular feedback
Tw20	tween 20
Tw80	tween 80
UV	ultraviolet
v/v	volume by volume
W	water
w/v	weight by volume
w/w	weight by weight
WME	warm microemulsion
w/o/w	water in oil in water
ZP	zeta potential