การตั้งสูตรตำรับยาเหน็บทวารหนักชนิดเจลแข็งในร่างกาย ซึ่งผสมสารสกัดบัวบกในรูปผงพ่นแห้งร่วมกับไคโตซาน

นางสาวมาศวลัย ลิขิตธนเศรษฐ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชกรรม ภาควิชาเภสัชกรรม
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2549

ISBN 974-14-2725-5

FORMULATION OF *IN SITU* GELLING SUPPOSITORY CONTAINING *CENTELLA ASIATICA* EXTRACT SPRAY-DRIED WITH CHITOSAN

Miss Masvalai Likitthanaset

A Thesis Submitted Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmaceutics
Department of Pharmacy
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2006

ISBN 974-14-2725-5

Thesis Title	Formulation of *in situ* gelling suppository containing *Centella asiatica* extract spray-dried with chitosan
By	Miss Masvalai Likitthanaset
Field of Study	Pharmaceutics
Thesis Advisor	Associate Professor Suchada Chutimaworapan, Ph.D.

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

.....Pornpen Pramyothin.....Dean of the Faculty of Pharmaceutical Sciences

(Associate Professor Pornpen Pramyothin, Ph.D.)

THESIS COMMITTEE

.....Porntip Nimmannitya..... Chairman

(Associate Professor Porntip Nimmannitya, M.Sc. in Pharm.)

.....Suchada Chutimaworapan..... Thesis Advisor

(Associate Professor Suchada Chutimaworapan, Ph.D.)

.....Waraporn Suwakul..... Member

(Associate Professor Waraporn Suwakul, Ph.D.)

.....Nontima V..... Member

(Assistant Professor Nontima Vardhanabhuti, Ph.D.)

..... Member

(Mukdavan Prakobvaitayakit, Ph.D.)

มาศวลัย ลิขิตธนเศรษฐ์: การตั้งสูตรตำรับยาเหน็บทวารหนักชนิดเจลแข็งในร่างกายซึ่งผสมสารสกัดบัวบกในรูปผงพ่นแห้งร่วมกับไคโตซาน (FORMULATION OF *IN SITU* GELLING SUPPOSITORY CONTAINING *CENTELLA ASIATICA* EXTRACT SPRAY-DRIED WITH CHITOSAN) อ. ที่ปรึกษา: รศ.ดร.สุชาดา ชุติมาวรพันธ์ 184 หน้า. ISBN 974-14-2725-5.

ยาเหน็บทวารหนักชนิดเจลแข็งในร่างกายผสมสารสกัดบัวบกพ่นแห้งร่วมกับไคโตซาน ได้เตรียมขึ้นด้วยสารผสมของพอล็อกซาเมอร์ โดยใช้การทดลองแบบแฟคตอเรียลศึกษาปัจจัยห้าชนิด ที่มีผลต่อเปอร์เซ็นต์ปริมาณผลผลิตและเปอร์เซ็นต์ความชื้นของผลิตภัณฑ์ที่ได้จากการพ่นแห้งสารสกัดบัวบก ซึ่งปัจจัยเหล่านี้คำนึงถึงปัจจัยด้านสูตรตำรับ (เปอร์เซ็นต์ของแข็ง, เปอร์เซ็นต์สารเติมแต่ง และอัตราส่วนระหว่างพอลิเมอร์ต่อสารสกัด) และปัจจัยด้านการพ่นแห้ง (อุณหภูมิอากาศเข้า และอัตราการป้อนสาร) สภาวะที่ต้องการคือได้ผลผลิตมากที่สุดในขณะที่ความชื้นน้อยที่สุด โดยนำเอาการออกแบบแฟคตอเรียลชนิดลำดับส่วนชนิด 2^{5-1} มาใช้เป็นขั้นตอนแรกของการคัดกรอง ซึ่งพบว่าปัจจัยที่มีนัยสำคัญทางสถิติมากที่สุดคือ อุณหภูมิอากาศเข้า และเปอร์เซ็นต์ของแข็ง ($P<0.01$) จากนั้นหาสภาวะออฟติมัมด้วยวิธีพื้นผิวตอบสนองโดยวิธีการออกแบบส่วนประกอบกลาง พบว่าโมเดลกำลังสองฟิตกับเปอร์เซ็นต์ปริมาณผลผลิต และโมเดลเชิงเส้นฟิตกับเปอร์เซ็นต์ความชื้น ($P<0.05$) จากนั้นหาบริเวณออฟติมัมโดยใช้กราฟ และใช้สูตรที่ออฟติมัมนี้ทำซ้ำๆเพื่อทดสอบกระบวนการการพ่นแห้ง พบว่าค่าเฉลี่ยของเปอร์เซ็นต์ปริมาณผลผลิตและเปอร์เซ็นต์ความชื้นอยู่ในช่วงระดับความเชื่อมั่น 95 เปอร์เซ็นต์ แสดงว่าโมเดลฟิตได้ดีกับผลการทดลอง ไมโครสเฟียร์ที่ได้ในขั้นตอนออฟติไมเซชันมีประสิทธิภาพในการบรรจุของสารเอเชียติโคไซด์ และมาเดคาสโซไซด์สูง (84.25±1.01 เปอร์เซ็นต์ และ 86.64±0.54 เปอร์เซ็นต์ ตามลำดับ) ขนาดอนุภาคเล็ก (5.19±0.02 ไมโครเมตร) ประจุบวก (32.87±1.39 มิลลิโวลท์) และคุณสมบัติการยึดติดเยื่อเมือกดี ผลจากดิฟเฟอเรนเชียลสแกนนิงแคลอรีเมทรี และพาวเดอร์เอ็กซ์เรย์ดิฟแฟรคชันแสดงให้เห็นว่าโมเลกุลของสารสกัดบัวบกกระจายในไคโตซาน และ/หรืออยู่ในรูปอสัณฐานแบบของแข็งกระจายตัว ให้การปลดปล่อยสารสำคัญเร็ว การเตรียมยาเหน็บชนิดเจลแข็งในร่างกายด้วยส่วนผสมพอล็อกซาเมอร์ พบว่าส่วนผสมพอล็อกซาเมอร์ 407 (18 เปอร์เซ็นต์) และพอล็อกซาเมอร์ 188 (4 เปอร์เซ็นต์) เป็นของเหลวที่อุณหภูมิห้องแต่เป็นเจลในช่วงอุณหภูมิที่กำหนดคือ 30-36 องศาเซลเซียส มีความแข็งของเจลและเวลาการแข็งตัวเหมาะสม การวิเคราะห์กลไกการปลดปล่อยของสารเอเชียติโคไซด์ และมาเดคาสโซไซด์ พบว่าการปลดปล่อยเป็นสัดส่วนกับรากที่สองของเวลา แสดงได้ว่าสารเอเชียติโคไซด์ และมาเดคาสโซไซด์อาจจะปลดปล่อยออกจากยาเหน็บโดยการแพร่แบบฟิกเกียน

ภาควิชา	เภสัชกรรม	ลายมือชื่อนิสิต.....
สาขาวิชา	เภสัชกรรม	ลายมือชื่ออาจารย์ที่ปรึกษา.....
ปีการศึกษา	2549	

4776595433 : MAJOR PHARMACEUTICS
KEY WORD: SPRAY DRYING / CENTELLA EXTRACT / FACTORIAL DESIGN / MICROSPHERES CHARACTERIZATION / LIQUID SUPPOSITORY / POLOXAMER

MASVALAI LIKITTHANASET: FORMULATION OF *IN SITU* GELLING SUPPOSITORY CONTAINING *CENTELLA ASIATICA* EXTRACT SPRAY-DRIED WITH CHITOSAN. THESIS ADVISOR: ASSOC. PROF. SUCHADA CHUTIMAWORAPAN, Ph.D., 184 pp. ISBN 974-14-2725-5.

The *in situ* gelling suppositories containing *Centella asiatica* extracts spray-dried with chitosan were formulated with poloxamer mixtures in this investigation. An experimental factorial design was built to investigate the effects of five parameters on percentage production yield and percentage moisture content of spray-dried products. These factors concerned both formulation parameters (%solid content, %additive concentration and polymer: extract ratio) and spray drying parameters (inlet temperature and feed rate). The operating condition was to maximize production yields while minimizing moisture contents. First screening experiments consisting of 2^{5-1} fractional factorial design revealed the most significant factors to be inlet temperature and %solid content ($P < 0.01$). Then, the optimal operating condition was estimated by response surface methodology. Central rotational composite design showed a quadratic model for %yield and a linear model for %moisture content were adequate ($P < 0.05$). The optimum region by overlay plot was carried out using the last condition to evaluate the repeatability of the spray-drying technique. The observed means obtained for yield and %moisture content were in range of the prediction intervals at 95% confidence level. The result clearly showed that the model fitted the experimental data well. The optimal microsphere formulation showed high loading efficiency of asiaticoside and madecassoside (84.25±1.01% and 86.64±0.54%, respectively), small size (5.19±0.02 μm), positive charge (32.87±1.39 mV) and good mucoadhesive property. Differential scanning calorimetry and powder x-ray diffraction studies revealed that the centella extract was molecularly dispersed with chitosan or/and existed in an amorphous state as a solid dispersion. The release from the optimal microspheres was fast. To prepare a novel *in situ* gelling suppositories with poloxamer mixtures, the mixtures of poloxamer 407 (18%) and poloxamer 188 (4%) existed as a liquid at room temperature, but gelled at 30–36°C with suitable gel strength and setting time. The analysis of release mechanism showed that the releases of asiaticoside and madecassoside were proportional to the square root of time, indicating that asiaticoside and madecassoside might be released from the suppositories by Fickian diffusion.

Department : Pharmacy Student's Signature: Masvalai .....
Field of Study : Pharmaceutics Advisor's Signature: Suchada Chutimaworapan .....
Academic Year : 2006

ACKNOWLEDGEMENTS

This study was carried out at the Faculty of Pharmaceutical Sciences, Department of Pharmacy, Chulalongkorn University.

I wish to express my sincere gratitude to my advisor Associate Professor Suchada Chutimaworapan, Ph.D. for suggesting the main topic of this study and for providing excellent working facilities. I am most grateful for her scientific guidance as well as for her constant enthusiasm and encouragement, all of which made the completion of this study possible.

I thank most sincerely the reviewers of this thesis, Associate Professor Porntip Nimmannitya, M.Sc. in Pharm, the chairman of my thesis examination committee, as well as other committee members. I am grateful to Associate Professor Waraporn Suwakul, Ph.D., Assistant Professor Nontima Vardhanabhuti, Ph.D. and Mukdavan Prakobvaitayakit, Ph.D. for their constructive criticism and for giving me valuable suggestions for its improvement.

My sincere thanks are due to all staff members of Department of Pharmacy and Department of Manufacturing Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University for helping and other persons whose names have not been mentioned here for their assistance and encouragement.

I am grateful to all my colleagues at the Faculty of Pharmaceutical Sciences for creating friendly atmosphere in which to work, and for sharing my moments of success and despair.

I wish to express appreciation for fully financial support of the research grant from the National Research Council of Thailand (NRCT).

Finally, greatest thank to my parents for their everlasting love, understanding, encouragement, and continued support during the course of my education.

CONTENTS

Page

THAI ABSTRACT iv

ENGLISH ABSTRACT v

ACKNOWLEDGEMENTS vi

CONTENTS vii

LIST OF TABLES viii

LIST OF FIGURES xiv

LIST OF ABBREVIATIONS xix

CHAPTER

I INTRODUCTION 1

II LITERATURE REVIEW 3

Hemorrhoids 4

Botanical, Chemical and Pharmacological Aspects of *Centella asiatica* (Linn.) 7

Mucoadhesive Chitosan Microspheres 11

In situ Forming Gel (Temperature Sensitive System): Poloxamer 17

The Release Mechanism of Controlled Release System 20

III MATERIALS AND METHODS 23

Materials 23

Apparatus 24

Methods 26

IV RESULTS AND DISCUSSION 45

Experimental Designs 45

Evaluation of Physicochemical Properties of Spray Dried Microspheres 63

Determination of Liquid Suppositories 96

Stability Study of Chitosan Microspheres Containing Centella Extract 108

V CONCLUSIONS 114

REFERENCES 117

APPENDICES 126

VITA 184

LIST OF TABLES

Table		Page
1	Parameters of spray drying process variables	30
2	Matrix of experiments of the fractional experimental design	30
3	Central composite design matrix of two parameters	32
4	Compositions of the liquid suppository bases	37
5	Fractional factorial design matrix of five parameters and the observed responses	46
6	ANOVA for selected factorial model of %yield	48
7	ANOVA for selected factorial model of %moisture content	48
8	Coefficients of the regression equation linking the responses to the experimental factors and major interactions (coded units)	49
9	Central composite design matrix of two parameters and the observed responses	53
10	ANOVA for Response Surface Quadratic Model of % yield	55
11	ANOVA for Response Surface Linear Model for %moisture content	55
12	Coefficients of the regression equation linking the responses to the experimental factors and major interactions (coded units)	56
13	The optimum region by overlay plot of two parameters and the observed responses	61
14	Observed responses and 95% CI (confidence interval)of optimization formulation	61
15	The particle size distributions of the spray-dried microspheres	73
16	Zeta potentials of spray-dried centella microspheres and some compounds	76
17	The adhesion times of microspheres adhered on mucus surface of pig rectum	77
18	The percentages of centella extract contents of spray-dried product	87
19	Coefficients of determination of the relationship between log percent remained versus time (first order)	95
20	Gelation temperatures of poloxamer solutions (n=5)	97
21	Gel strengths of poloxamer solutions (n=5)	99

Table **Page**

22 Effect of centella microspheres on the physical properties of poloxamer base 100

23 Release of asiaticoside and madecassoside from pure centella extract with the variation of base suppositories (n=3) 102

24 Release of asiaticoside and madecassoside from spray-dried centella extract with chitosan with the variation of base suppositories (n=3)..... 102

25 $T_{80\%}$ of asiaticoside and madecassoside depended on the types of base and forms of active..... 105

26 Fitting results of the asiaticoside and madecassoside release data from all suppositories..... 106

27 Percentages labeled amount of asiaticoside and madecassoside in optimal microspheres in stability test 111

28 Data for calibration curve of asiaticoside and madecassoside in mobile phase..... 128

29 Data for calibration curve of asiaticoside and madecassoside in 50% ethanol..... 128

30 The percentages of analytical recovery of asiaticoside and madecassoside in mobile phase..... 135

31 The percentages of analytical recovery of asiaticoside and madecassoside in 50% ethanol..... 135

32 Data of within run precision of asiaticoside and madecassoside in mobile phase..... 136

33 Data of within run precision of asiaticoside and madecassoside in 50% ethanol..... 137

34 Data of between run precision of asiaticoside and madecassoside in mobile phase..... 137

35 Data of between run precision of asiaticoside and madecassoside in 50% ethanol..... 137

36 Fractional factorial design matrix of five parameters and the viscosity and outlet temperature..... 138

37 Fractional factorial design matrix of five parameters and the moisture content (n=3)..... 139

Table **Page**

38 Fractional factorial design matrix of five parameters and the particle size of microspheres (n=3)..... 140

39 Central composite design matrix of two parameters and the viscosity and outlet temperature..... 141

40 Central composite design matrix of two parameters and the moisture content (n=3)..... 141

41 The optimum region by overlay plot of two parameters and the viscosity and outlet temperature..... 142

42 The zeta potential of microspheres (n=3)..... 142

43 Gelation temperatures of poloxamer solutions (n=5)..... 143

44 Gel strengths of poloxamer solutions (n=5)..... 143

45 Effect of centella microspheres on the physical properties of poloxamer base (n=3)..... 144

46 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F1)..... 172

47 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F2)..... 172

48 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F3)..... 172

49 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F4)..... 173

50 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F5)..... 173

51 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F6)..... 173

52 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F7)..... 174

53 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F8)..... 174

54 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F9)..... 174

55 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F10)..... 175

Table **Page**

56 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F11)..... 175

57 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F12)..... 175

58 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F13)..... 176

59 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F14)..... 176

60 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F15)..... 176

61 Percentages of asiaticoside and madecassoside released from chitosan microspheres (F16)..... 177

62 Percentages of asiaticoside and madecassoside released from chitosan microspheres (optimal)..... 177

63 Percentages of asiaticoside and madecassoside released from chitosan microspheres (centella extract)..... 177

64 Percentages of asiaticoside (pure centella extract) released from Suppocire®AM conventional suppository..... 178

65 Percentages of madecassoside (pure centella extract) released from Suppocire®AM conventional suppository..... 178

66 Percentages of asiaticoside (pure centella extract) released from polyethylene glycol (PEG) conventional suppository..... 178

67 Percentages of madecassoside (pure centella extract) released from polyethylene glycol (PEG) conventional suppository..... 179

68 Percentages of asiaticoside (pure centella extract) released from poloxamer liquid suppository..... 179

69 Percentages of madecassoside (pure centella extract) released from poloxamer liquid suppository..... 179

70 Percentages of asiaticoside (optimal microspheres) released from Suppocire®AM conventional suppository..... 180

71 Percentages of madecassoside (optimal microspheres) released from Suppocire®AM conventional suppository..... 180

Table **Page**

72 Percentages of asiaticoside (optimal microspheres) released from polyethylene glycol (PEG) conventional suppository..... 180

73 Percentages of madecassoside (optimal microspheres) released from polyethylene glycol (PEG) conventional suppository..... 181

74 Percentages of asiaticoside (optimal microspheres) released from poloxamer liquid suppository..... 181

75 Percentages of madecassoside (optimal microspheres) released from poloxamer liquid suppository..... 181

76 ANOVA for selected factorial model (%yield)..... 182

77 ANOVA for selected factorial model (%moisture content)..... 182

78 ANOVA for Response Surface Quadratic Model (%yield)..... 182

79 Coefficient for Response Surface Quadratic Model (%yield)..... 183

80 ANOVA for Response Surface Linear Model (%moisture content)..... 183

81 Coefficient for Response Linear Model (%moisture content)..... 183

LIST OF FIGURES

Figure		Page
1	Formation of hemorrhoids	4
2	*Centella asiatica* (Linn.) Urban	8
3	Some structures of the triterpenoids from *Centella asiatica* (Linn.)	8
4	Structure of chitosan	11
5	Interaction of mucin with chitosan; showing mucin as long strands attached to chitosan aggregates. Chitosan interacts with mucin through charge interaction and hydrogen-bonding mechanisms	13
6	Variations of microparticle formulations	14
7	Structure of poloxamer	18
8	Spray dryer (SD-06, Labplant, Ltd., UK)	29
9	Central composite design for two variables	31
10	Mucoadhesive property testing apparatus (assembled in the laboratory	34
11	Gelation temperature testing apparatus (assembled in the laboratory)	39
12	Gelation temperature testing apparatus; top view (assembled in the laboratory)	40
13	Schematic illustration of gel strength-measuring device (assembled in the laboratory)	41
14	Gel strength device	41
15	Gel strength testing apparatus (assembled in the laboratory)	42
16	The measuring of setting time of poloxamer solution	42
17	The sugar tube prepared for filling liquid suppositories	43
18	Main effect plots for % yield and %moisture content (factorial)	50
19	A scattered plot between outlet temperature and moisture content on spray-dried microspheres	51
20	Influence of additive (Aerosil®) on percentage yields in collector	52
21	Response surface plot of % yield and linear regression plot of %moisture content	58
22	The normal probability plots of the %yield and %moisture content	59

Figure **Page**

23 The optimum region by overlay plot of two responses (%yield and %moisture content) evaluated as a function of inlet temperature and %solid content..... 60

24 Powders of optimal formulation from optimization design.....62

25 Scanning electron photomicrographs of raw materials.....65

26 Scanning electron photomicrographs of microspheres prepared without additive in formulation.....66

27 Scanning electron photomicrographs of microspheres prepared from experimental design (F1, F2 and F3)..... 67

28 Scanning electron photomicrographs of microspheres prepared from experimental design (F4, F5 and F6)..... 68

29 Scanning electron photomicrographs of microspheres prepared from experimental design (F7, F8 and F9).....69

30 Scanning electron photomicrographs of microspheres prepared from experimental design (F10, 11 and F12)..... 70

31 Scanning electron photomicrographs of microspheres prepared from experimental design (F13, F14 and F15).....71

32 Scanning electron photomicrographs of microspheres prepared from experimental design (F16 and optimal).....72

33 A scattered plot between %moisture content and appearance particle size of spray-dried microspheres.....74

34 A scattered plot between viscosity of spray solution and appearance particle size of spray-dried microspheres.....75

35 The photographs of mucoadhesive microspheres adhered on mucus surface of pig rectum at difference time..... 79

36 The photographs of mucoadhesive microspheres adhered on mucus surface of pig rectum at difference time (continued).....80

37 The DSC thermograms of standard madecassoside, standard asiaticoside, centella extract, physical mixture of centella extract with chitosan, centella microspheres, chitosan microspheres and optimal formulation..... 82

38 The DSC thermograms of F1-F8..... 83

39 The DSC thermograms of F9-F16..... 84

Figure **Page**

40 X-ray diffractograms of standard asiaticoside, standard madecassoside, centella extract, the physical mixture of centella extract with chitosan, chitosan microspheres, optimal microspheres and F1-16.....86
41 Dissolution profiles of asiaticoside and madecassoside (pure centella extract, F1 and F2).....89
42 Dissolution profiles of asiaticoside and madecassoside (F3, F4 and F5)....90
43 Dissolution profiles of asiaticoside and madecassoside (F6, F7 and F8)....91
44 Dissolution profiles of asiaticoside and madecassoside (F9, F10 and F11)..92
45 Dissolution profiles of asiaticoside and madecassoside (F12, F13 and F14)93
46 Dissolution profiles of asiaticoside and madecassoside (F15, F16 and optimal).....94
47 The suppositories for in vitro release analyse.....101
48 Release profiles of asiaticoside and madecassoside from pure centella extract in suppositories prepared with; Suppocire® AM, PEGs and poloxamer.....103
49 Release profiles of asiaticoside and madecassoside from spray-dried centella extract in suppositories prepared with; Suppocire® AM, PEGs and poloxamer.....104
50 DSC thermograms of optimal formulation microspheres after stress condition (40° C, 75% RH).....109
51 X-ray diffractograms of optimal formulation microspheres after stress condition (40° C, 75% RH).....110
52 Hydrolysis of asiaticoside by hydronium ion.....112
53 Hydrolysis of asiaticoside by hydroxide ion.....113
54 HPLC chromatograms of standard stock solution.....129
55 HPLC chromatograms of mixtures standard stock solution and centella total triterpenes extract.....130
56 HPLC chromatograms of mobile phase and 50% ethanol.....131
57 HPLC chromatograms of blank microspheres in suppositories.....132
58 Standard curves of asiaticoside and madecassoside in mobile phase by HPLC method.....133
59 Standard curves of asiaticoside and madecassoside in 50% ethanol by HPLC method.....134

Figure **Page**

60 Zeta potential of chitosan.....145
61 Zeta potential of ethylcellulose.....145
62 Zeta potential of Aerosil®.....146
63 Zeta potential of F1..... 146
64 Zeta potential of F2.....147
65 Zeta potential of F3.....147
66 Zeta potential of F4..... 148
67 Zeta potential of F5..... 148
68 Zeta potential of F6..... 149
69 Zeta potential of F7..... 149
70 Zeta potential of F8..... 150
71 Zeta potential of F9..... 150
72 Zeta potential of F10..... 151
73 Zeta potential of F11..... 151
74 Zeta potential of F12..... 152
75 Zeta potential of F13..... 152
76 Zeta potential of F14..... 153
77 Zeta potential of F15..... 153
78 Zeta potential of F16..... 154
79 Zeta potential of optimal..... 154
80 Size and size distribution of F1..... 155
81 Size and size distribution of F2..... 156
82 Size and size distribution of F3..... 157
83 Size and size distribution of F4..... 158
84 Size and size distribution of F5..... 159
85 Size and size distribution of F6..... 160
86 Size and size distribution of F7..... 161
87 Size and size distribution of F8..... 162
88 Size and size distribution of F9..... 163
89 Size and size distribution of F10..... 164
90 Size and size distribution of F11..... 165
91 Size and size distribution of F12..... 166
92 Size and size distribution of F13.....167

Figure **Page**

93 Size and size distribution of F14..... 168

94 Size and size distribution of F15.....169

95 Size and size distribution of F16..... 170

96 Size and size distribution of optimal formulation..... 171

LISTS OF ABBREVIATIONS

ANOVA	=	analysis of variance
°C	=	degree Celsius
CCD	=	Central Composite Design
cm	=	centimeter
CV	=	coefficient of variation
df	=	degree of freedom
DSC	=	differentials scanning calorimetry
et al.	=	*et alii*, 'and others'
g	=	gram
hr	=	hour
HPLC	=	high performance liquid chromatography
kv	=	kilovolt
M	=	Molar
mg	=	milligram
min	=	minute
ml	=	milliliter
mm	=	millimeter
mPas	=	millipascal
MW	=	molecular weight
n	=	sample size
PEG	=	polyethylene glycol
pH	=	the negative logarithm of the hydrogen ion concentration
R^2	=	coefficient of determination
RH	=	relative humidity
rpm	=	round per minute
s	=	second
SD	=	standard deviation
TECA	=	titrated extract of *Centella asiatica*
TTF	=	total triterpenic fraction
TTFCA	=	total triterpenoid fraction of *Centella asiatica*
µg	=	microgram

µm	=	micrometer
USP	=	The United States Pharmacopoeia National Formulary
UV	=	ultraviolet
w/v	=	weight by volume
w/w	=	weight by weight