TABLE OF CONTENTS

	Page
Acknowledgement	iii
Abstract (English)	iv
Abstract (Thai)	vi
List of tables	xi
List of illustrations	xvi
Abbreviations and symbols	xx
Chapter 1 Introduction and literature survey	1
A. Introduction	1
1.1 Statement and significance of the problem	1
1.2 Objective	3
1.3 Scope of study	3
B. Literature survey	3
1.1 Tranexamic acid	3
1.2 Liposomes	10
1.3 Tranexamic acid entrapped in liposomes	33
Chapter 2 Experimental	35
2.1 Material and equipments	35
2.1.1 Chemicals	35
2.1.2 Equipments	35
2.2 Methods	36
2.2.1 Preparation of liposomes	36
2.2.2 Physical properties study of liposomes formulation	38
2.2.3 Quantitative analysis of tranexamic acid	39
2.2.4 Determination of tranexamic acid encapsulation efficacy in liposome	40

2.2.5 Physical and chemical stability study of tranexamic acid in liposome formulations 41
2.2.6 The release study of tranexamic acid from liposomes 42

Chapter 3 Results 44
3.1 Characteristics of liposome formulations 44
3.1.1 The physical appearances of liposome formulations 44
3.1.2 pH measurement of liposome formulations 46
3.1.3 Particle size and particle size distribution of liposome 47
3.1.4 Morphology of liposome formulations determination by transmission electron microscope 54
3.1.5 Thermal analysis of liposome formulations 56
3.1.5.1 Glass transition temperature 56
3.1.5.2 Transition temperature and enthalpy of transition 65
3.2 Quantitative analysis of tranexamic acid 66
3.2.1 Standard curve of tranexamic acid 66
3.2.2 Validation of the assay of tranexamic acid by a spectrophotometric method 75
3.3 Determination of the percentages of tranexamic acid entrapped in liposome formulations 76
3.4 Stability study of tranexamic acid entrapped in liposome formulations 79
3.4.1 Physical stability 79
3.4.2 Chemical stability 85
3.5 The release study of tranexamic acid from solution and liposome of various liposome formulations 107

Chapter 4 Discussion 117
4.1 Physico-chemical characteristics of liposome formulations 117
4.1.1 The physical appearances of liposome formulations 117
4.1.2 pH measurement of liposome formulations 117
4.1.3 Particle size and particle size distribution of liposome 118

4.1.4 The morphology of liposome formulations 119
4.1.5 Thermal analysis of liposome formulations 119
4.2 Quantitative analysis of tranexamic acid by a spectrophotometric method 121
4.3 The percentages of tranexamic acid entrapment in liposome formulations 123
4.4 Physical and chemical stability of tranexamic acid in liposome formulations 124
4.4.1 Physical stability of tranexamic acid in liposome 124
4.4.2 Chemical stability of tranexamic acid in liposome 129
4.5 The release study of tranexamic acid from solution and liposome of various liposome formulations 128

Chapter 5 Conclusion 131
Reference 133
Appendices 141
Curriculum vitae 161

LIST OF TABLES

Table		Page
1.1	The following dosages are recommended for impaired renal function (moderate to severe) patients (Drug Facts and Comparison, 1999)	8
1.2	Liposome-based products or under development (Andrew, 1999)	11
1.3	Liposome classifications (Talsm, 1994)	16
1.4	Techniques used for characterization of liposomes (Janoff, 1999 ; Swarbrick, ; Boylan, 1994)	24
1.5	Quality control assays of liposomal formulation (Janoff, 1999 ; Swarbrick, ; Boylan,1994)	26
2.1	The compositions (g) of nine liposome formulations (in 60 ml DI water)	37
3.1	The physical appearances of twelve liposome formulations which were freshly prepared after 24 hrs	44
3.2	The pH values of six liposome formulations comparing to DI water, 5%TA solution and 10%TA solution	46
3.3	Mean particle size (μm) of six liposome formulations	47
3.4	The glass transition temperature (^{0}C) of phosphatidylcholine, cholesterol, stearylamine, dicetyl phosphate, TA 7:2:1(10%TA, +) and 7:2:1(10%TA, -) liposome formulations.	56
3.5	The transition temperature (^{0}C) of phosphatidylcholine, cholesterol, stearylamine, dicetyl phosphate, TA 7:2:1(+),7:2:1(5%TA,+), 7:2:1(10%TA, +) 7:2:17:2:1(-), (5%TA, -),7:2:1(10%TA, -) liposome formulations	65
3.6	The enthalpy of transition (ΔHJ/g) of Emulmetik 950®, cholesterol, stearylamine, dicetyl phosphate, TA 7:2:1(+),7:2:1(5%TA,+), 7:2:1(10%TA, +) 7:2:17:2:1(-), (5%TA, -),7:2:1(10%TA, -) liposome formulations	66

3.7	Absorbance (λ = 415 nm) of various concentrations of the reference standard TA (Lot.1)	72
3.8	Absorbance (λ = 415 nm) of various concentrations of the reference standard TA (Lot.2)	72
3.9	Absorbance (λ = 415 nm) of various concentrations of the reference standard TA (Lot.3)	73
3.10	Absorbance (λ = 415 nm) from tripicate analysis of the reference standard TA	73
3.11	Comparison of the percentages recovery of TA determination by spectrophotometric and potentiometric titration methods	75
3.12	Percentage recovery of TA in liposome formulations by determination a spectrophotometric method	75
3.13	Amount of TA (mg) in supernatant of the 1 g liposome formulations	76
3.14	Amount of TA (mg) in pellet of the 1 g liposome formulations	77
3.15	Percentages of TA entrapped in liposomes from liposome Formulations	77
3.16	Percentages loading of TA in various liposome formulations	78
3.17	The physical appearance of 7:2:1(+), 7:2:1(5%TA,+), 7:2:1(10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -)liposome formulations when freshly prepared	79
3.18	The physical appearance of 7:2:1(+), 7:2:1(5%TA,+), 7:2:1(10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -) liposome formulations when kept at 4 $\pm$ 1 , 30 $\pm$ 1 and 45 $\pm$ 1 ^{0}C for 30 days	80
3.19	The physical appearance of 7:2:1(+), 7:2:1(5%TA,+), 7:2:1(10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -) liposome formulations when kept at 4 $\pm$ 1 , 30 $\pm$ 1 and 45 $\pm$ 1 ^{0}C for 60 days	80
3.20	The physical appearance of 7:2:1(+), 7:2:1(5%TA,+), 7:2:1(10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -) liposome formulations when kept at 4 $\pm$ 1 , 30 $\pm$ 1 and 45 $\pm$ 1 ^{0}C for 90 days	81

3.21	The remaining amounts of TA in the 7:2:1(5%TA,+) liposome formulation when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60, and 90 days	86
3.22	The remaining amounts of TA in the 7:2:1(10%TA,+) liposome formulation when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60, and 90 days	87
3.23	The remaining amounts of TA in the 7:2:1(5%TA,-) liposome formulation when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60, and 90 days	88
3.24	The remaining amounts of TA in the 7:2:1(10%TA,-) liposome formulation when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60, and 90 days	89
3.25	Comparison of the percentages of the remaining total TA in liposome formulations when kept at 4 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	90
3.26	Comparison of the percentages of the remaining total TA in liposome formulations when kept at 30 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	91
3.27	Comparison of the percentages of the remaining total TA in liposome formulations when kept at 45 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	92
3.28	Calculation of the degradation rate (slope) and the shelf life of the liposme formulations	93
3.29	The predicted shelf life of the liposome formulations	94
3.30	The total amounts of TA in the 7:2:1(5%TA,+) liposome formulation 1 g when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days	95
3.31	The total amounts of TA in the 7:2:1(10%TA,+) liposome formulation 1 g when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days	96
3.32	The total amounts of TA in the 7:2:1(5%TA,-) liposome formulation 1 g when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days	97
3.33	The total amounts of TA in the 7:2:1(10%TA,-) liposome formulation 1 g when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days	98
3.34	Comparison of the percentages of the total TA in liposome formulations 1 g when kept at 4 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	99
3.35	Comparison of the percentages of the total TA in liposome formulations 1 g when kept at 30 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	100

3.36	Comparison of the percentages of the total TA in liposome formulations 1 g when kept at 45 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	101
3.37	Determination of chemical kinetic of the percentages of total TA in liposome of liposome formulations fitted in zero order, first order and Higuchi model	102
3.38	Comparison of the percentages of the total TA in pellet of liposome formulations 1 g when kept at 4 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	103
3.39	Comparison of the percentages of the total TA in pellet of liposome formulations 1 g when kept at 30 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	104
3.40	Comparison of the percentages of the total TA in pellet of liposome formulations 1 g when kept at 45 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	105
3.41	Determination of chemical kinetic of the percentages of total TA in pellet in liposomes of liposome formulations fitted in zero order, first order and Higuchi model	106
3.42	Percentages cumulative TA released from 7:2:1 (5%TA,+) liposome formulation at 0 to 24 hrs in 12 ml of receiver medium	108
3.43	Percentages cumulative TA released from 7:2:1 (10%TA,+ liposome formulation at 0 to 24 hrs in 12 ml of receiver medium	108
3.44	Percentages cumulative TA released from 7:2:1 (5%TA,-) liposome formulation at 0 to 24 hrs in 12 ml of receiver medium	109
3.45	Percentages cumulative TA released from 7:2:1 (10A,- liposome formulation at 0 to 24 hrs in 12 ml of receiver medium	109
3.46	Mean percentages cumulative TA released from 7:2:1 liposome formulation at 0 to 24 hrs in 12 ml of receiver medium	110
3.47	Percentages cumulative traneamic acid released from 5% solution at 0 to 24 hrs in 12 ml of receiver medium	111
3.48	Percentages cumulative traneamic acid released from 10% solution at 0 to 24 hrs in 12 ml of receiver medium	112
3.49	Mean percentages cumulative traneamic acid released from 5% and 10% solution at 0 to 24 hrs in 12 ml of receiver medium	112
3.50	Mean Percentages cumulative TA released from Liposome formulations and solution at 0 to 24 hrs in 12 ml of receiver medium	113

3.51	Determination of kinetic of the cumulative percentages of TA release from liposome formulations and solution fitted in zero order, first order and Higuchi model	115
3.52	The releasing rate of the percentages cumulative amount of TA released fitted with Higuchi model and total TA released at 24 hrs from the liposome formulations and solution	116
A1	Charge molecules of H_3O^+ and OH^-	143
C1	The percentages of TA remaining in liposomes at 4, 30 and 45 ^{0}C	145
C2	The values of C_o (intercept), k (slope) and r^2 of 7:2:1(5%TA,+)	146
D1	Size distribution (μm) of the 7:2:1 (+) liposome formulation Lot.1	149
D2	Size distribution (μm) of the 7:2:1 (+) liposome formulation Lot.2	150
D3	Size distribution (μm) of the 7:2:1 (5%TA,+) liposome formulation Lot.1	151
D4	Size distribution (μm) of the 7:2:1 (5%TA+) liposome formulation Lot.2	152
D5	Size distribution (μm) of the 7:2:1 (10%TA,+) liposome formulation Lot.1	153
D6	Size distribution (μm) of the 7:2:1 (10%TA+) liposome formulation Lot.2	154
D7	Size distribution (μm) of the 7:2:1 (-) liposome formulation Lot.1	155
D8	Size distribution (μm) of the 7:2:1 (-) liposome formulation Lot.2	156
D9	Size distribution (μm) of the 7:2:1 (5%TA,-) liposome formulation Lot.1	157
D10	Size distribution (μm) of the 7:2:1 (5%TA-) liposome formulation Lot.2	158
D11	Size distribution (μm) of the 7:2:1 (10%TA,-) liposome formulation Lot.1	159
D12	Size distribution (μm) of the 7:2:1 (10%TA-) liposome formulation Lot.2	160

LIST OF ILLUSTRATIONS

Figure		Page
1.1	Structure of TA (Kathlem, 1999)	5
1.2	The multiple concentric lipid bilayers of a multilamellar liposome are clearly indicated by the freeze-fracture electron micrograph.	14
1.3	TEM photomicrograph of multilamellar liposomes following negative staining with phosphotungstic acid (Benita, 1996)	15
1.4	Morphology of different liposome structures (Barenholz, 1994)	18
1.4	Diagram showing the key steps in liposome formulation by the REV (reverse-phase evaporation) technique.	21
1.5	Interrelationships of factors to consider in the design of pharmaceutical liposome preparations (Lichtenberg, 1988)	25
3.1	Physical appearances of freshly prepared liposome formulations from left to right : 7:2:1 (+), 7:2:1, (5%,+), 7:2:1, (10%,+), 7:2:1 (-), 7:2:1, (5%,-) and 7:2:1, (10%,-) respectively	45
3.2	Particle size (μm) distribution of 7:2:1 (+) Lot.1	48
3.3	Particle size (μm) distribution of 7:2:1 (+) Lot.2	48
3.4	Particle size (μm) distribution of 7:2:1 (5%TA,+) Lot.1	49
3.5	Particle size (μm) distribution of 7:2:1 (5%TA,+) Lot.2	49
3.6	Particle size (μm) distribution of 7:2:1 (10%TA,+) Lot.1	50
3.7	Particle size (μm) distribution of 7:2:1 (10%TA,+) Lot.2	50
3.8	Particle size (μm) distribution of 7:2:1 (-) Lot.1	51
3.9	Particle size (μm) distribution of 7:2:1 (-) Lot.2	51
3.10	Particle size (μm) distribution of 7:2:1 (5%TA, -) Lot.1	52
3.11	Particle size (μm) distribution of 7:2:1 (5%TA, -) Lot.2	52
3.12	Particle size (μm) distribution of 7:2:1 (10%TA, -) Lot.1	53

3.13	Particle size (μm) distribution of 7:2:1 (10%TA, -) Lot.2	53
3.14	The morphology and lamellarity of 7:2:1 liposome formulation, 6000x	54
3.15	The morphology and lamellarity of 7:2:1 (5%TA,+) liposome formulation, 5000x	55
3.16	The morphology and lamellarity of 7:2:1 (5%TA,-) liposome formulation, 6000x	55
3.17	The glass transition temperatures (^{0}C) of Emulmetik 950® (1), cholesterol (2), stearylamine (3), dicetyl phosphate (4), TA, 7:2:1(10%TA, +) (5), and 7:2:1(10%TA, -) (6) liposome formulations	57
3.18	The glass transition temperatures (^{0}C) of Emulmetik 950®	58
3.19	The glass transition temperatures (^{0}C) of cholesterol	59
3.20	The glass transition temperatures (^{0}C) of stearylamine	60
3.21	The glass transition temperatures (^{0}C) of dicetyl phosphate	61
3.22	The glass transition temperatures (^{0}C) of TA	62
3.23	The glass transition temperatures (^{0}C) of 7:2:1(10%TA, +)	63
3.24	The glass transition temperatures (^{0}C) of 7:2:1(10%TA, -)	64
3.25	DSC Thermograms of Emulmetik 950® (1), cholesterol (2), stearylamine (3), dicetyl phosphate (4), and TA (5).	67
3.26	DSC Thermograms of (7:2:1,+) (1), and 7:2:1 (5%TA,+) (2)	68
3.27	DSC Thermograms of (7:2:1,+) (1), and 7:2:1 (10%TA,+) (2)	69
3.28	DSC Thermograms of (7:2:1,-) (1), and 7:2:1 (5%TA,-) (2)	70
3.29	DSC Thermograms of (7:2:1,-) (1), and 7:2:1 (10%TA,-) (2)	71
3.30	The standard curve of standard TA in DI water	74
3.31	The physical appearance of the prepared liposome formulations when stored at 4 ± 1 ^{0}C for 90 days from left to right were 7:2:1(+), 7:2:1(5%TA,+), 7:2:1 (10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -)	82
3.32	The physical appearance of the prepared liposome formulations when stored at 30 ± 1 ^{0}C for 90 days from left to right were 7:2:1(+), 7:2:1(5%TA,+), 7:2:1 (10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -)	83
3.33	The physical appearance of the prepared liposome formulations when stored at 45 ± 1 ^{0}C for 90 days from left to right were 7:2:1(+), 7:2:1(5%TA,+), 7:2:1 (10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -)	84

3.34.	Comparison of the percentages of the remaining total TA in liposome formulations when kept at 4 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	90
3.35	Comparison of the percentages of the remaining total TA in liposome formulations when kept at 30 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	91
3.36	Comparison of the percentages of the remaining total TA in liposome formulations when kept at 45 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days	92
3.37	The histograms of the predicted shelf life (days) at 4 ± 1 ^{0}C, 30 ± 1 ^{0}C and 45 ± 1 ^{0}C of liposome formulations.	94
3.38	The comparison of the percentages of the total TA in liposome formulation when kept at 4 ± 1 ^{0}C at 0, 14, 30, 60 and 90 days	99
3.39	The comparison of the percentages of the total TA in liposome formulation when kept at 30 ± 1 ^{0}C at 0, 14, 30, 60 and 90 days	100
3.40	The comparison of the percentages of the total TA in liposome formulation when kept at 45 ± 1 ^{0}C at 0, 14, 30, 60 and 90 days	101
3.41	The comparison of the percentages of the total TA in pellet of liposome formulation 1 g when kept at 4 ± 1 ^{0}C at 0, 14, 30, 60 and 90 days	103
3.42	The comparison of the percentages of the total TA in pellet of liposome formulation 1 g when kept at 30 ± 1 ^{0}C at 0, 14, 30, 60 and 90 days	104
3.43	The comparison of the percentages of the total TA in pellet of liposome formulation 1 g when kept at 45 ± 1 ^{0}C at 0, 14, 30, 60 and 90 days	105
3.44	Percentages Cumulative TA released from the liposome formulations at 0 to 24 hrs	110
3.45	Mean percentages cumulative traneamic acid released from 5% and 10% solution	112
3.46	Percentages cumulative TA released from Liposome formulations and solution at 0 to 24 hrs	113
3.47	Reaction of TA with 2,4,6 trinitrobenzosulfonic acid in alkaline medium (Atmaca,1989)	122

3.48 Hydrolysis reactions of phosphatidylcholine in aqueous liposome dispersions (Grit, 1993) 125

3.49 The effect of pH on the hydrolysis of saturated soybean phosphatidylcholine (Grit, 1993) 126

3.50 The effect of temperature on the hydrolysis of saturated soybean phosphatidylcholine 127

ABBREVIAIONS AND SYMBOLS

7:2	Neutral liposome composed of 7:2 molar ratio of Emulmetik 950® / cholesterol, without the entrapped TA
7:2 (5%TA)	Neutral liposome composed of 7:2 molar ratio of Emulmetik 950® / cholesterol, with the entrapped 5%TA
7:2 (10%TA)	Neutral liposome composed of 7:2 molar ratio of Emulmetik 950® / cholesterol, with the entrapped 10%TA
7:2:1 (+)	Positively charged liposome of 7:2:1 molar ratio of Emulmetik 950® / cholesterol / stearylamine, without the entrapped TA
7:2:1 (5%TA,+)	Positively charged liposome of 7:2:1 molar ratio of Emulmetik 950® / cholesterol / stearylamine, with the entrapped 5%TA
7:2:1 (10%TA,+)	Positively charged liposome of 7:2:1 molar ratio of Emulmetik 950® / cholesterol / stearylamine, with the entrapped 10%TA
7:2:1 (-)	Negatively charged liposome of 7:2:1 molar ratio of Emulmetik 950® / cholesterol / stearylamine, without the entrapped TA
7:2:1 (5%TA,-)	Negatively charged liposome of 7:2:1 molar ratio of Emulmetik 950® / cholesterol / stearylamine, with the entrapped 5%TA
7:2:1 (10%TA,-)	Negatively charged liposome of 7:2:1 molar ratio of Emulmetik 950® / cholesterol / stearylamine, with the entrapped 10%TA
CHL	Cholesterol
DCP	Dicetyl phosphate
DSC	Differential scanning calorimetry
HSC	Hydrogenated soya phosphatidylcholine
SA	Stearylamine
TA	Tranexamic acid
TEM	Transmission electron microscope