

Ain Shams University

Faculty of Pharmacy

Department of Pharmaceutics and Industrial Pharmacy

Improvement of the Bioavailability of Buspirone Hydrochloride using Intranasal Delivery Systems

By

Hamza Nimr Mahmoud Bshara

Bachelor of Pharmaceutical Sciences, May 2009, An-Najah National University Nablus-Palestine

A thesis submitted in the partial fulfillment of the requirements for the master degree of

Pharmaceutical Sciences (Pharmaceutics)

Under the supervision of

Prof. Dr. Abd El Hameed Abdallah El-Shamy

Professor of Pharmaceutics and Industrial Pharmacy

Faculty of Pharmacy - Ain Shams University

Prof. Dr. Samar Mansour Holayel

Dr. Rihab Osman Ahmed

Professor of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy - Ain Shams University

Lecturer of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy - Ain Shams University



Acknowledgement

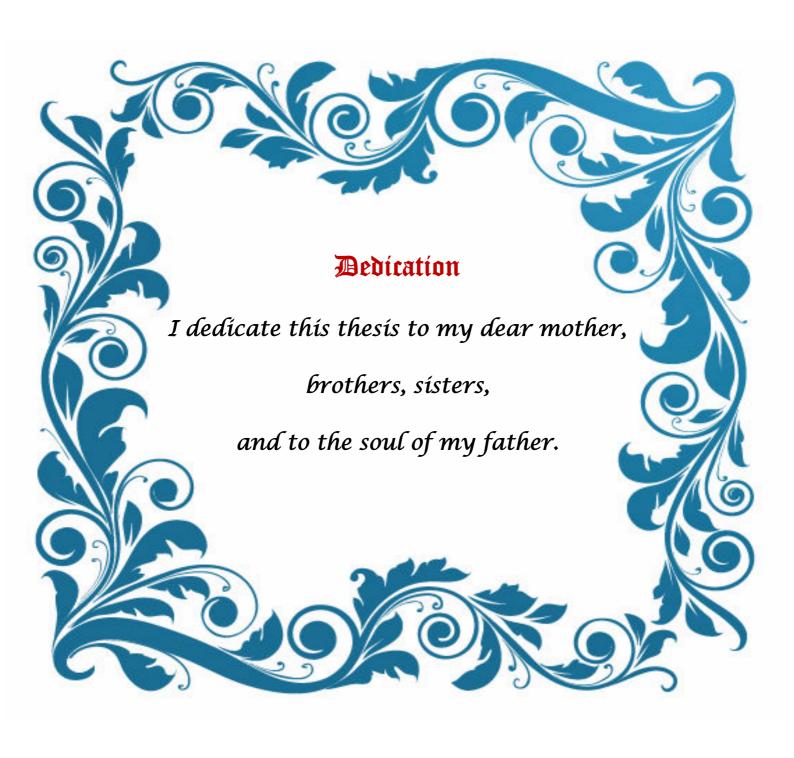
I am deeply thankful to Allah by the grace of which the present work was realized.

I would like to express my best regards and appreciation to **Prof.Dr. Abd El Hameed Abdallah El-Shamy**, Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, for his instructive supervision, proving facilities, continuous advice and continuous encouragement.

I would like to express my deep and sincere gratitude to **Prof.Dr. Samar Mansour Holayel**, Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, for suggesting the point, planning the work, her instructive discussion, ultimate advice and her great effort devoted toward the completion of this thesis.

I am also grateful to **Dr. Rihab Osman Ahmed**, Lecturer of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, for her teaching on microemulsion science, valuable advice, support and efforts for this work, careful and exigent reading of the thesis, the critical comments, suggestions and corrections. She has become a mentor and an example for my academic life.

I would like to thank the members of the department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University.



Description	Page
List of abbreviations	I
List of tables	\mathbf{V}
List of figures	IX
Abstract	XIV
General introduction	
Introduction	1
Anatomy and physiology of the nose in humans	1
Nasal epithelium	3
Respiratory region	3
Olfactory region	4
Nasal secretion	6
Factors influencing the absorption of drugs across the nasal epithelium	7
Physiological barrier	7
Mucociliary clearance.	7
Enzymes	8
Physicochemical characteristics of the drug.	8
Challenges in transmucosal nasal drug delivery	9
Nasal pharmaceutical dosage form	10
Microemulsions	11
Microemulsions as intranasal drug delivery	13
Buspirone hydrochloride	15
Scope of work	19

Chapter I:	Preparation and characterization of microemulsion
	systems for intranasal delivery of buspirone HCl

Introduction	21
Experimental	31
Materials	31
Equipment	31
Methodology	33
UV spectrophotometric scanning of buspirone hydrochloride in	
different media	33
Construction of buspirone hydrochloride calibration curves in the	
different media	33
Determination of the saturation solubility of buspirone hydrochloride	
in different oils	34
Construction of pseudo-ternary phase diagram	34
Identification tests for the type of microemulsions	35
Dilution test	35
Conductivity measurement	35
Characterization of unloaded microemulsion formulations	35
Polarized light microscopy	35
Percent transmittance	35
Optical clarity: Refractive index	36
Determination of the droplet size and polydispersity index	36
Viscosity	36
Determination of the pH	37
Statistical analysis	37
Results and discussion.	38
UV spectrophotometric scanning of buspirone hydrochloride in	
different media	38

Procedural constants of buspirone hydrochloride in the different media	38
Saturation solubility of buspirone hydrochloride in different oils	43
Phase behavior	45
Type of the microemulsions	54
Dilution test.	54
Conductivity measurement.	54
Characterization of unloaded microemulsion formulations	54
Polarized light microscopy	54
Percent transmittance.	55
Optical clarity: Refractive index	55
Particle size and polydispersity index	55
Viscosity	56
pH	57
Conclusion.	62
Chapter II: Formulation and in-vitro evaluation of buspirone HCl	
intranasal microemulsion systems	
Introduction	63
Experimental	73
Materials	73
Equipment	73
Methodology	75
Preparation of buspirone HCl loaded microemulsion systems	75
Characterization of buspirone HCl loaded microemulsion	77
Drug content	77
Viscosity	77
Measurement of mucoadhesive strength	77
Fourier transform infrared spectroscopy (FTIR)	7 9
Transmission electron microscopy (TFM)	79

In-vitro drug release study	80
Ex-vivo drug permeation study	80
Physical stability study	81
Statistical analysis	81
Results and discussion.	82
Characterization of buspirone HCl loaded microemulsion systems	82
Drug content	82
Viscosity	82
Mucoadhesion performance evaluation	83
Fourier transform infrared spectroscopy (FTIR)	85
Transmission electron microscopy (TEM)	91
In-vitro drug release study	93
Ex-vivo drug permeation Study	96
Physical stability study	99
Conclusion	103
Chapter III: Pharmacokinetics and brain distribution study on selected	
intranasal buspirone HCl formulae	
Introduction	105
Direct systemic delivery.	111
Intravenous drug delivery to the brain	111
Intra-arterial delivery	111
Intranasal drug delivery to the brain	112
Experimental	114
Materials	114
Equipment	114
Animals	115
Methodology	116

In-vivo pharmacokinetic and brain distribution study	116
Animals, dosing and administration.	118
Sample preparation for analysis	119
Preparation of standards	119
Plasma sample preparation	119
Brain sample preparation	119
Chromatographic conditions	120
Calibration range, linearity, limit of detection, limit of quantitation for	
buspirone HCl in rat plasma and brain	120
Assay validation	121
Recovery	121
Precision and accuracy	121
Stability studies	121
Pharmacokinetic analysis	122
Transport study using the in-vivo rat model	124
Histopathological study	125
Statistical analysis	125
Results and discussion.	126
HPLC method validation	126
Selectivity and specificity	126
Calibration range, linearity, limit of detection, limit of quantitation for	
buspirone HCl in rat plasma and brain	128
Assay validation	130
Recovery	130
Precision and accuracy	133
Stability studies	135
In-vivo pharmacokinetic and brain distribution study	138
Plasma pharmacokinetic parameters	138

Transport study using the in-vivo rat model
Histopathological study
Conclusion
Ethical committee
Summary
References
Appendix
Arabic summary

List of Abbreviations

AB Absolute bioavailability

AE Absorption enhancer

AME Absorptive-mediated transport

ANOVA Analysis of variance

AUC Area under the curve

BBB Blood brain barrier

BCS Biopharmaceutical classification system

BCSFB Blood cerebrospinal fluid barrier

BH Buspirone hydrochloride

BHME Buspirone hydrochloride microemulsion

BHMME Buspirone hydrochloride mucoadhesive microemulsion

BHS Buspirone hydrochloride solution

cAMP Cyclic-adenosine monophosphate

CD Cyclodextrin

CD-BHMME Buspirone hydrochloride mucoadhesive microemulsion containing

hydroxypropyl-β-cyclodextrin

CD-MME Mucoadhesive microemulsion containing hydroxypropyl-β-cyclodextrin

cm Centimeter

cm² Centimeter square

C_{max} Maximum concentration of drug

CNS Central nervous system

cp Centipoise

CPP Critical packing parameter

CSF Cerebro-spinal fluid

CTAB Hexadecyl (cetyl) trimethyl ammonium bromide

CV% Percent coefficient of variation

^o C Celsius temperature

Da Dalton

DDAB Didodcecylammonium bromide

DILT Diltiazem

DNA Deoxyribonucleic acid

DTE% Drug targeting efficiency

DTP% Nose-to-brain direct transport percentage

ECF Extracellular fluid

FDA Food and drug administration

FTIR Fourier transform infrared spectroscopy

GIT Gastrointestinal tract

GRAS Generally regarded as safe

Grp Group

HLB Hydrophile-lipophile balance

HP-β-CD Hydroxypropyl-β-cyclodextrin

HPLC High performance liquid chromatography

hrs Hour

IA Intra-arterial

ICH International conference on harmonization

IN Intranasal

IPM Isopropyl myristate

IS Internal standard

IV Intravenous

IVS Intravenous solution

K Procedural constant

KDa Kilo Dalton

kg Kilogram

Labr Labrafac lipophile® WL 1349

LOD Limit of detection

LOQ Limit of quantitation

μ**g** Microgram

μl Microliter

μ**m** Micrometer

g Gram

m² Meter square

MCC Mucociliary clearance

ME Microemulsion

mg Milligram

min Minute

ml Milliliter

mm Millimeter

MME Mucoadhesive microemulsion

MRP Multi drug resistance associated protein

MRT Mean residence time

Mw Molecular weight

NA Not applicable

ng Nanogram

nm Nanometer

O/W Oil-in-water

PB Phosphate buffer

PBS Phosphate buffer saline

PEG Polyethylene glycol

PG Propylene glycol

P-gp P-glycoprotein

PIT Phase inversion temperature

pKa Ionization constant

PTS Peptide transport system

QC Quality control

R² Coefficient of determination

RB Relative bioavalability

rpm Round per minute

S mix Surfactant/cosurfactant mixture

s/n Signal-to-noise ratio

SD Standard deviation

SE Standard error

 $t_{1/2}$ Half life

T80 Tween[®] 80

TEM Transmission electron microscopy

 T_{max} Time of occurrence for maximum drug concentration

UV Ultraviolet

W/O Water-in-oil

w/w Weight per weight

v/v Volume per volume

List of Tables

Table	75. 1.1. N	ъ
No.	Table Name	Page
1	Relation between concentration and absorbance of buspirone	40
	hydrochloride in water at 238 nm.	
2	Relation between concentration and absorbance of buspirone	41
	hydrochloride in phosphate buffer (pH 6.8).	
3	Relation between concentration and absorbance of buspirone	42
	hydrochloride in isopropyl alcohol.	
4	Composition and formation ability of microemulsion systems used for	48
	phase diagram construction.	
5	Composition of the prepared microemulsions.	53
6	Physicochemical parameters of selected microemulsion formulations.	58
7	Composition of buspirone HCl loaded microemulsion systems for	76
	intranasal delivery.	
8	Physicochemical properties of the prepared buspirone HCl loaded	84
	microemulsion systems.	
9	OH band frequencies of unloaded and buspirone HCl loaded	86
	microemulsion formulations.	
10	<i>In-vitro</i> release of buspirone hydrochloride from solution and	94
	microemulsion formulations.	
11	Ex-vivo release of buspirone hydrochloride from solution and	97
	microemulsion formulations.	
12	Results of the stability studies of loaded microemulsion.	100
13	Results of the stability studies of loaded mucoadhesive microemulsion.	101