



Enhancement of bioavailability of a poorly soluble drug

*A thesis submitted for partial fulfillment of the requirements for the
Master Degree of Pharmaceutical Sciences
(Pharmaceutics)*

By

Marwa Elsayed Mohammed

*Bachelor of Pharmaceutical Sciences, June 2009, Ain Shams University
Teaching assistant, department of Pharmaceutics and Industrial
Pharmacy, Faculty of Pharmacy, Ain Shams University*

Under supervision of

Prof. Nahed Daoud Mortada

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

Prof. Gehanne Abd El-Samie Awad

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

Dr. Rihab Osman Ahmed

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

**Department of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy
Ain Shams University**

2019

Acknowledgements

First of all thanks to *GOD*, by the grace of whom, this work was successfully achieved.

I would like to express my hearty appreciation to *Prof. Nahed Daoud Mortada*, Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, for her instructive supervision and encouragement throughout the work.

I am greatly thankful to *Prof. Gehanne Abd El-Samie Awad*, Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, for her fruitful supervision and kind help throughout the work.

No words could ever express my deep thanks to *Dr. Rihab Osman Ahmed*, Associate Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, as my mentor and my role model, for the great help and effort she devoted for the completion of this thesis.

Deep thanks to *Prof. Adel Bekeer*, Professor of Pathology, Faculty of Medicine, Cairo University, for helping in the histopathology part of this thesis and also *DR. Abdelrahman Hassan*, ophthalmology specialist, Faculty of Medicine, Alfayoum University, for his help during *in vivo* study.

I would like to express my deep thanks to all my *Colleagues* in the Department of Pharmaceutics and Industrial Pharmacy for their support.

I am profoundly grateful to all *members of my family (my father, my mother, my sisters (Enas, Doaa, Sara and Nada) and my brother (Mohammed))* who supported and helped me. Extending gratitude to my dear husband (*Adel*) and my daughter (*Remas*) for their sincere support and encouragement during the work.

Marwa Elsayed Mohammed

LIST OF CONTENTS

Item	Page number
List of abbreviations.	I
List of tables.	V
List of figures.	VIII
Abstract.	XV
General introduction.	1
Scope of work.	11
Chapter I: Preparation and characterization of acetazolamide nanosuspensions	13
Introduction.	14
Experimental.	22
Materials.	22
Equipment.	23
Methodology.	24
(1) Experimental design.	24
(2) Preparation of different acetazolamide nanosuspensions.	25
(2.1) Preparation of ACZ-NS using a single stabilizer.	25
(2.1.1) Experimental variables.	27
(2.1.1.1) Effect of stirring rate and time.	27
(2.1.1.2) Effect of sonication type and time.	27
(2.1.2) Formulation variables.	27
(2.1.2.1) Effect of solvent to antisolvent ratio (S/AS).	27
(2.1.2.2) Effect of ACZ concentration.	27
(2.1.2.3) Effect of stabilizer type and concentration.	28
(2.2) Preparation of ACZ-NS using a binary stabilizer.	28
(2.2.1) Combination of PVA with soya lecithin (SL).	29
(2.2.1.1) Effect of soya lecithin concentration.	29

(2.2.1.2) Effect of probe sonication power and time.	29
(2.2.2) Combination of PVA with polymers.	29
(2.2.2.1) Cationic polymers.	29
(2.2.2.2) Anionic polymers.	30
(2.3) Preparation of polymer treated ACZ-NS stabilized with PVA/SL.	30
(2.3.1) Preparation of chitosan treated ACZ-NS stabilized with PVA/SL.	30
(2.3.2) Preparation of Y/PG treated ACZ-NS stabilized with PVA/SL.	30
(2.4) Maximizing ACZ-NS loading efficiency.	31
(3) Characterization of ACZ-NS.	32
(3.1) Particle size, polydispersity index and zeta potential analysis.	32
(3.2) Microscopical examination.	32
(3.2.1) Transmission electron microscopy (TEM).	32
(3.2.2) Optical microscopy.	32
(3.3) Solid state characterization.	32
(3.3.1) Differential scanning calorimetry (DSC).	32
(3.3.2) X-ray powder diffraction (XRPD).	33
(3.4) Saturation solubility determination.	33
(3.4.1) UV scanning of ACZ in deionized water.	34
(3.4.2) Calibration curve construction of ACZ in deionized water	34
(3.5) Removal of DMSO from ACZ-NS.	34
(3.6) Stability study.	35
(4) Statistical analysis.	35
Results and discussion.	36

(1) Effect of different variables during preparation of ACZ-NS using a single stabilizer.	37
(1.1) Experimental variables.	37
(1.1.1) Effect of stirring rate and time.	37
(1.1.2) Effect of sonication type and time.	39
(1.2) Formulation variables.	42
(1.2.1) Effect of solvent to antisolvent ratio (S/AS).	42
(1.2.2) Effect of ACZ concentration.	44
(1.2.3) Effect of type and concentration of stabilizer.	45
(2) ACZ-NS prepared with a binary stabilizer.	49
(2.1) ACZ-NS prepared with PVA/SL.	49
(2.1.1) Effect of soya lecithin concentration.	49
(2.1.2) Effect of probe sonication power and time.	51
(2.2) ACZ-NS prepared with PVA/polymer.	53
(2.2.1) Cationic polymer (CS).	53
(2.2.2) Anionic polymers (Y/ PG).	56
(3) ACZ-NS stabilized with PVA/SL and treated with different polymers.	58
(3.1) Chitosan- treated ACZ-NS stabilized with PVA/SL.	59
(3.2) Anionic polymers-treated ACZ-NS stabilized with PVA/SL.	60
(4) Optimization of ACZ-NS loading efficiency.	62
(5) Characterization of selected ACZ-NS formulae.	63
(5.1) Microscopical examination of NS using TEM.	63
(5.2) Differential scanning calorimetry (DSC) analysis.	65
(5.3) X-ray powder diffraction (XRPD).	73
(5.4) ACZ saturation solubility.	73
(5.4.1) Determination of λ_{\max} in deionized water.	73
(5.4.2) Calibration curve of ACZ in deionized water.	75

(5.5) Removal of organic solvent (DMSO) from ACZ-NS.	76
(5.6) Stability study.	78
Conclusions.	80
Chapter II: Preparation and characterization of spray dried acetazolamide nanosuspensions	82
Introduction.	83
Experimental.	90
Materials.	90
Equipment.	91
Methodology.	92
(1) Experimental design.	92
(2) Preparation of spray dried ACZ nanosuspensions (ACZ-SDN).	92
(3) Characterization of ACZ-SDN.	94
(3.1) Yield.	94
(3.2) ACZ association efficiency (AE) and drug loading (DL).	94
(3.2.1) UV scanning of ACZ in different media.	94
(3.2.2) Calibration curve of ACZ in different media.	94
(3.2.3) ACZ association efficiency (AE) and drug loading (DL).	95
(3.3) Determination of redispersibility index of ACZ-SDN.	95
(3.4) Detection of residual DMSO in SDN using proton magnetic resonance (^1H NMR) technique.	96
(3.5) Differential scanning calorimetry (DSC).	96
(3.6) X-ray powder diffraction (XRPD).	96
(3.7) Morphological examination of ACZ-SDN.	97
(3.8) Determination of moisture content of ACZ-SDN.	97
(3.9) Effect of different tonicity adjusting agents on the	97

characteristics of the reconstituted SDN.	
(3.10) Microscopical examination of redispersed SDN.	98
(3.11) <i>In vitro</i> release of ACZ from ACZ-SDN.	98
(3.12) Sterilization study.	99
(3.12.1) Sterility test of sterilized ACZ-SDN.	99
(3.12.2) Effect of gamma radiation on physicochemical properties of ACZ-SDN.	99
(3.13) Stability studies.	99
(4) Statistical analysis.	100
Results and discussion.	101
(1) Spectrophotometric assay of ACZ in different media.	101
(1.1) UV scanning of ACZ in different media.	101
(1.2) Calibration curve of ACZ in different media.	102
(2) Spray drying of ACZ-NS.	103
(2.1) Effect of spray drying process parameters.	103
(2.2) Effect of carrier composition.	105
(2.3) Effect of nanoparticles: carrier ratio.	109
(2.4) Effect of stabilizing polymer.	111
(3) Characterization of selected ACZ-SDN formulae.	114
(3.1) Differential scanning calorimetry (DSC).	114
(3.2) X-ray powder diffraction (XRPD).	120
(3.3) Morphological examination of ACZ-SDN using SEM.	126
(3.4) Moisture content of ACZ-SDN.	131
(3.5) Effect of different tonicity adjusting agents	132
(3.6) TEM microscopical examination following ACZ-SDN redispersion.	133
(3.7) <i>In vitro</i> release study of ACZ-SDN.	135
(3.8) Sterilization of selected ACZ-SDN formulae.	136

(3.8.1) Sterility test.	137
(3.8.2) Effect of sterilization on physicochemical properties of ACZ-SDN.	137
(3.9) Stability studies.	139
Conclusions.	143
Chapter III: Safety and biological activity of ocular spray dried acetazolamide nanosuspensions	145
Introduction.	146
Experimental.	149
Materials.	149
Animals.	149
Equipment.	150
Methodology.	150
Animal handling.	150
(1) Evaluation of ocular tolerance of ACZ-SDN.	150
(2) Evaluation of <i>in vivo</i> ocular hypotensive efficacy of ACZ- SDN.	152
(2.1) Glaucoma induction in rabbits (Ocular hypertensive model).	153
(2.2) Experimental design.	153
(3) Statistical analysis.	154
Results and discussion	155
(1) Evaluation of ocular tolerance of ACZ-SDN.	155
(2) Evaluation of <i>in vivo</i> ocular hypotensive efficacy of ACZ-SDN	160
Conclusions.	166
General conclusions.	167
Future perspectives.	168
Summary.	169

References.	176
--------------------	-----

Appendix.

Ethical committee approval for *in vivo* studies.

Arabic summary.

LIST OF ABBREVIATIONS

Abbreviation	Designation
ACZ	Acetazolamide.
AE	Association efficiency.
ANOVA	One-way analysis of variance.
APIs	Active pharmaceutical ingredients.
AS	Antisolvent.
AS-PT	Antisolvent precipitation.
AUC	Area under the curve.
BAB	Blood aqueous barrier.
BCS	Biopharmaceutical classification system.
BRB	Blood retinal barrier.
C	Carrier
C_{∞}	Bulk solubility.
CA	Carbonic anhydrase.
CAG	Closed angle glaucoma.
CAIs	Carbonic anhydrase inhibitors.
CD44	Cluster of differentiation 44.
CMC	Critical micelle concentration.
Conc	Concentration.
cP	Centipoise.
CS	Chitosan.
Cs	Saturation solubility.
Da	Dalton.
DL	Drug loading.
DLS	Dynamic light scattering.
DMSO	Dimethyl sulfoxide.
DSC	Differential scanning calorimetry.

f_2	Similarity factor.
FD	Freeze drying.
FDA	Food& drug administration.
GRAS	Generally regarded as safe.
h	Hours.
HLB	Hydrophilic- lipophilic balance.
^1H NMR	Proton nuclear magnetic resonance.
HPH	High pressure homogenization.
ICH	International Conference on Harmonization.
I_{irr}	Overall ocular irritation index.
IOP	Intraocular pressure.
IR	Infrared.
KGy	Kilogray.
KHz	Kilo Hertz.
Kv	Kilovolt.
Leu	Leucine.
LMW	Low molecular weight.
LNS	Liquid nanosuspension.
M	Molar.
mA	Milli Amber.
Man	Mannitol.
mg	Milligram.
MHz	Mega Hertz.
min	Minutes.
mL	Milliliter.
mV	Millivolt.
MW	Molecular weight.
MWCO	Molecular weight cut off.

NaCl	Sodium chloride.
NaOH	Sodium hydroxide.
ND	Not determined.
nm	Nanometer.
NPs	Nanoparticles.
NS	Nanosuspension.
OAG	Open angle glaucoma.
OFAT	One factor at time.
P-407	Poloxamer 407.
PBS	Phosphate buffer saline.
PC	Phosphatidyl choline.
PDI	Polydispersity index.
PEG-400	Polyethylene glycol 400.
PG	Poly- γ -glutamic acid.
PM	Physical mixture.
ppt	Precipitation.
PS	Particle size.
PSD	Particle size distribution.
PVA	Polyvinyl alcohol.
RGCs	Retinal ganglion cells.
R^2	Coefficient of determination.
RT	Room temperature.
R_{index}	Redispersibility index.
S	Solvent.
s	Second.
s.d	Standard deviation.
SAA	Surfactant.
SD	Spray drying.

SDN	Spray dried nanosuspension.
SDP	Spray dried powder.
SE	Standard error.
SEM	Scanning electron microscope.
SL	Soya bean Lecithin.
SNS	Solid nanosuspension.
STF	Simulated tear fluid.
TDL	Theoretical drug loading.
TEM	Transmission electron microscope.
T _g	Glass transition temperature.
TGA	Thermogravimetric analysis.
T _{inlet}	Inlet temperature.
T _{outlet}	Outlet temperature.
UV	Ultraviolet.
WBM	Wet ball milling.
XRPD	X- ray powder diffraction.
Y	Sodium hyaluronate.
ζ	Zeta potential.
λ _{max}	Wavelength of maximum absorption.
μm	Micrometer.

LIST OF TABLES

Table Number	Table title	Page number
I	Modified Draize grading scale for clinical evaluation of ocular irritation.	152
1	Tested factors and compositions of ACZ-NS formulae prepared by AS-PT method using a single stabilizer.	26
2	Experimental variables and composition of ACZ-NS formulae prepared using binary stabilizers.	28
3	Composition of polymer treated ACZ-NS with different ACZ loadings.	31
4	Effect of experimental variables on the PS and PDI of ACZ-NS.	38
5	Effect of formulation variables on PS and PDI of ACZ-NS.	42
6	Characteristics of ACZ-NS formulae prepared with PVA/SL binary stabilizer.	50
7	Characteristics of ACZ-NS prepared with PVA with or without chitosan (CS).	55
8	Characteristics of ACZ-NS formulae prepared with PVA+/-anionic polymers.	57
9	Characteristics of chitosan-treated ACZ-NS stabilized with PVA/SL.	60

10	Characteristics of anionic polymer-treated ACZ-NS stabilized with PVA/SL.	61
11	Characteristics of different ACZ-NS formulae prepared at various drug loadings.	62
12	Saturation solubility of ACZ as received and in selected ACZ-NS in deionized water.	75
13	Effect of freeze drying on PS, PDI and ζ of ACZ-NS selected formulae.	78
14	Colloidal stability of ACZ-NS upon storage at 4°C for 4 days.	79
15	Process and formulation variables applied in the preparation of ACZ-SDN formulae.	93
16	Effect of different spray drying process parameters on ACZ-SDN characteristics.	105
17	Effect of leucine concentration on the characteristics of ACZ-SDN.	108
18	Effect of nanoparticles to carrier ratio on the characteristics of ACZ-SDN.	110
19	Effect of ACZ-NS composition on the characteristics of ACZ-SDN.	112
20	Moisture content of selected ACZ-SDN formulae.	131
21	Recovery characteristics of formula SA in different isotonicity adjusting agents.	133