



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

Preparation and Evaluation of Thermoreversible Injectable Drug Delivery Systems

Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of
Doctor of Philosophy in Pharmaceutical Sciences
(Pharmaceutics)

By

Hend Mohamed Abd el-Bar Hamad

Bachelor of Pharmaceutical Sciences, 2006, Ain Shams University
Master Degree in Pharmaceutics, 2011, Ain Shams University

Under the supervision of

Prof. Nahed Daoud Mortada Prof. Gehanne Abdel Samie Awad

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

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

Dr. Rihab Osman Ahmed

Dr. Amal youssef Mohamed

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

Researcher, Head of Pharmaceutics
Lab in NODCAR and lecturer of
Pharmaceutics in MUST University

**Cairo
2014**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

لَا يُلْقِيهِ اللَّهُ نَفْسًا إِلَّا أَوْشَعْمَا لَهَا مَا كَسَبَتْ
وَعَلَيْهَا مَا كَسَبَتْ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ
أَخْطَأْنَا رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِحْرَاءَ مَا حَمَلَتْهُ عَلَى
الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ
وَاعْمُرْ بَيْنَنَا وَبَيْنَ الْكَاذِبِينَ

(سورة البقرة- آية: ٢٨٦)

Acknowledgment

*Praise is to **ALLAH**, the most merciful and most gracious, by the help of whom this work has been completed.*

*I would like to express my deepest gratitude to **Prof. Dr Nahed Daoud Mortada**, Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, for her excellent guidance, caring, patience, support and warm advices.*

*I am in great debt to **Prof. Dr Gehanne Abdel Samie Awad**, Professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, for her constant encouragement, support, and invaluable suggestions made this work successful. She has been everything that one could want in supervisor. I deeply appreciate her valuable scientific supervision and continuous guidance.*

*I would like to note a special acknowledgement to **Dr Rihab Osman**, Lecturer of Pharmaceutics and Industrial Pharmacy, Faculty of pharmacy, Ain Shams University, for her great efforts, keen supervision, valuable guidance, continuous enforcement and generous support.*

*I am also grateful to **Dr Amal youssef**, Researcher of Pharmaceutics, head of Pharmaceutics lab in NODCAR, and Lecturer of Pharmaceutics and Industrial Pharmacy, Faculty of pharmacy, MUST University, for her valuable guidance, support and follow up for this work.*

*I would like to record my thankfulness to **Prof. Dr Adel Bakeer**, Professor of Pathology, Faculty of Veterinary Medicine, Cairo University, for his kind cooperation in conducting the histopathological investigations incorporated in this study.*

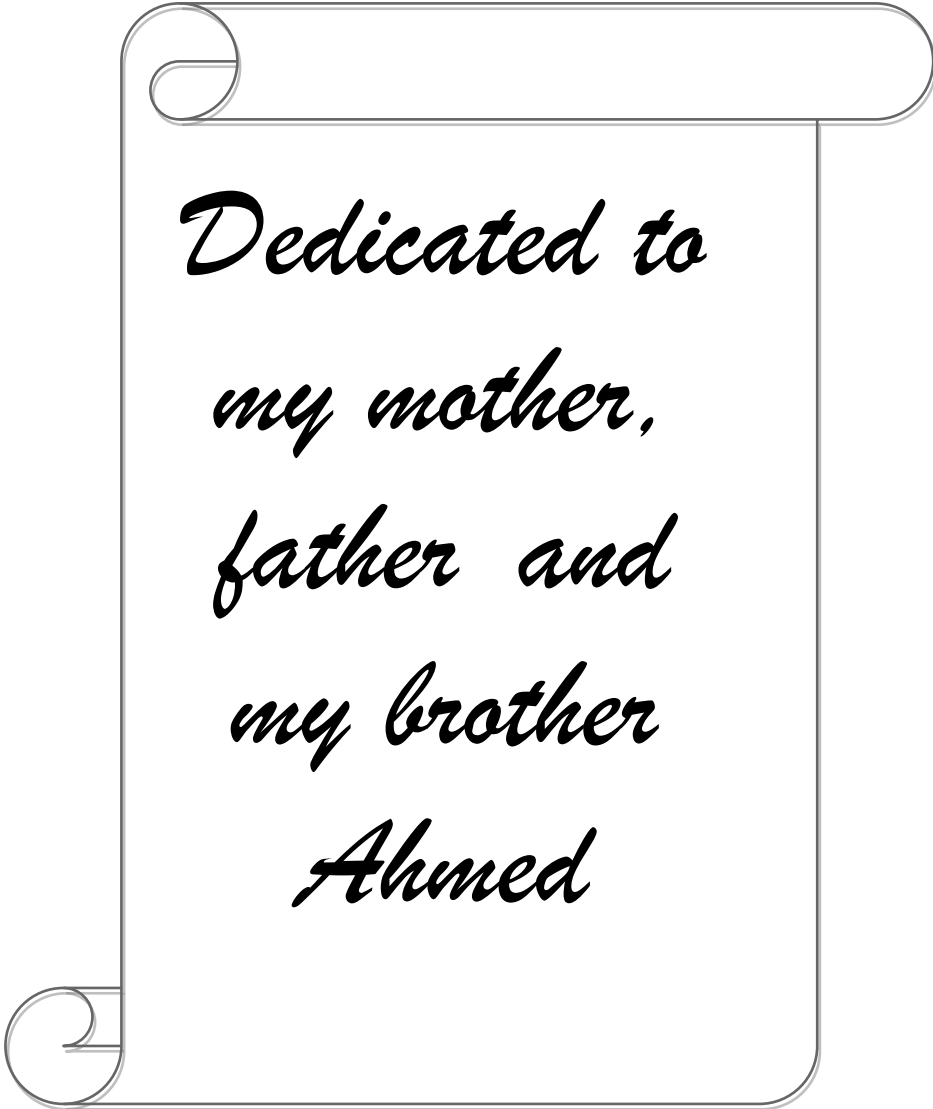
*Here is my chance to thank my best friend **Akram Mostafa** for his kindness, friendship, continuous encouragement, support and understanding in all my life.*

*I would like to extend my deep thanks to all the staff members and all my colleagues in the Department of Pharmaceutics, **NODCAR**.*

*I am deeply and forever indebted to **my beloved mother, father and my brother Ahmed** for their support, understanding, patience, love and encouragement during my whole life.*

Hend Mohamed Abdel-Bar

2014



*Dedicated to
my mother,
father and
my brother
Ahmed*

List of Contents

| <i>Title</i> | <i>Page</i> |
|--|-------------|
| List of Tables | ix |
| List of Figures | xii |
| List of Abbreviations | xx |
| Abstract | xxiv |
| General Introduction | 1 |
| Scope of Work | 17 |
| <i>Chapter I: Preparation and Characterization of Cisplatin Chitosan/β-Glycerophosphate Thermoreversible Hydrogels</i> | |
| Introduction | 19 |
| Experimental | 28 |
| Materials | 28 |
| Equipment | 28 |
| Methodology | 30 |
| Determination of Chitosan Degree of Deacetylation | 30 |
| Determination of First Derivative Spectrum of N-Acetyl Glucosamine | 30 |
| Construction of Calibration Curve of N-Acetyl Glucosamine | 30 |
| Calculation of Chitosan Degree of Deacetylation | 31 |
| Preparation and Characterization of Highly Deacetylated Chitosan | 32 |
| Preparation of Highly Deacetylated Chitosan | 32 |
| Characterization of Chitosan by FT-IR Spectroscopy | 33 |
| Preparation of Thermoreversible Cisplatin Loaded Chitosan / β -Glycerophosphate Hydrogel | 33 |
| Preparation of Plain Chitosan/ β - Glycerophosphate Thermoreversible Hydrogel | 33 |
| Fourier Transform Infra-Red Spectroscopy Analysis | 35 |

| | |
|--|----|
| Preparation of Cisplatin Loaded Chitosan/ β -Glycerophosphate Thermoreversible Hydrogel | 35 |
| Characterization of Cisplatin Thermoreversible Chitosan / β -Glycerophosphate Hydrogel | 36 |
| Determination of pH | 36 |
| Sol-to-gel Study | 36 |
| Morphological Study | 37 |
| <i>In-vitro</i> Cisplatin Release Study | 37 |
| Study of the Effect of pH on Cisplatin-Chitosan Complexation | 42 |
| Viscosity Measurement | 42 |
| Determination of Free Cisplatin | 42 |
| Fourier Transform Infra-Red Spectroscopy Analysis | 43 |
| Preparation and Characterization of pH Adjusted Cisplatin Chitosan / β -Glycerophosphate Thermoreversible Hydrogel | 43 |
| Preparation of pH Adjusted Cisplatin Loaded Chitosan / β -Glycerophosphate Thermoreversible Hydrogel | 43 |
| Characterization of pH Adjusted Cisplatin Chitosan/ β -Glycerophosphate Thermoreversible Hydrogel | 43 |
| Statistical Analysis | 44 |
| Results and Discussion | 45 |
| Chitosan Degree of Deacetylation | 45 |
| First Derivative Spectrum of N-Acetyl Glucosamine | 46 |
| Calibration Curve of N-Acetyl Glucosamine | 46 |
| Calculation of Chitosan Degree of Deacetylation | 48 |
| Preparation and Characterization of Highly Deacetylated Chitosan | 51 |
| Preparation of Highly Deacetylated Chitosan | 51 |
| Characterization for HMW Chitosan by FT-IR Spectroscopy | 52 |

| | |
|--|----|
| Preparation and Characterization of Cisplatin Thermoreversible Chitosan / β -Glycerophosphate Hydrogel | 55 |
| Preparation of Plain Chitosan/ β -Glycerophosphate Hydrogel | 55 |
| FT-IR spectra of Chitosan/ β -Glycerophosphate Thermoreversible Hydrogels | 66 |
| Effect of Drug Loading Method on Hydrogel Properties | 69 |
| Effect on pH | 69 |
| Effect on Gelation Temperature and Time | 70 |
| Effect on Morphology | 73 |
| <i>In-vitro</i> Cisplatin Release Study from Thermoreversible Chitosan/ β -Glycerophosphate Hydrogels | 78 |
| HPLC Assay of Cisplatin | 78 |
| Validation of the HPLC Method | 78 |
| <i>In-vitro</i> Drug Release Study | 82 |
| Effect of pH on Cisplatin-Chitosan Complexation | 87 |
| Viscosity Measurements | 89 |
| Free Cisplatin Concentration | 90 |
| FT-IR studies | 91 |
| Characterization of pH Adjusted Cisplatin Chitosan/ β -Glycerophosphate Thermoreversible Hydrogel | 93 |
| Gelation Temperature and Time | 93 |
| <i>In-vitro</i> Release of Cisplatin | 94 |
| Conclusions | 97 |

Chapter II: Preparation and Characterization of Cisplatin Microemulsion Based Autogel

| | |
|--------------|-----|
| Introduction | 101 |
| Experimental | 112 |
| Materials | 112 |
| Equipment | 112 |
| Methodology | 114 |

| | |
|---|-----|
| Preparation and Characterization of Plain Microemulsion | 114 |
| Construction of Pseudo-ternary Phase Diagram | 114 |
| Characterization of Plain Microemulsions | 114 |
| Determination of Plain Microemulsion Microstructure by Conductivity Measurement | 114 |
| Determination of Droplet Size and Zeta Potential of Plain Microemulsions | 115 |
| Preparation and Characterization of Cisplatin Loaded Microemulsions | 115 |
| Preparation of Cisplatin Loaded Microemulsions | 115 |
| Characterization of Cisplatin Loaded Microemulsions | 116 |
| Determination of Cisplatin Loaded Microemulsion Microstructure by Dynamic Electric Conductivity Measurement | 116 |
| Determination of Droplet Size, Polydispersity Index and Zeta Potential of Cisplatin Microemulsions | 117 |
| Transmission Electron Microscopy of Cisplatin Microemulsion | 117 |
| <i>In-vitro</i> Release of Cisplatin from Selected Microemulsion | 117 |
| Preparation and Characterization of Plain Microemulsion Based Autogel | 118 |
| Preparation of Plain Microemulsion Based Autogel | 118 |
| Characterization of Plain Microemulsion Based Autogel | 118 |
| Preparation and Characterization of Cisplatin Loaded Microemulsion Based Autogel | 119 |
| Preparation of Cisplatin Loaded Microemulsion Based Autogel | 119 |

| | |
|--|-----|
| Characterization of Cisplatin Loaded Microemulsion Based Autogel | 119 |
| Gelation Temperature and Time of Cisplatin Loaded Microemulsion Based Autogel | 119 |
| Viscosity Measurements of Cisplatin Loaded Microemulsion Based Autogel | 119 |
| Transmission Electron Microscopy of Cisplatin Loaded Microemulsion Based Autogel | 120 |
| Scanning Electron Microscopy of Cisplatin Loaded Microemulsion Based Autogel | 120 |
| In-vitro Cisplatin Release from Microemulsion Based Autogel | 120 |
| <i>In-vitro</i> Enzymatic Degradation | 120 |
| Physical Stability of Cisplatin Loaded Microemulsion Based Autogel | 121 |
| Gravitational Stability Study | 121 |
| Freeze Thaw Stress Stability Study | 122 |
| Thermal Stability Study | 122 |
| Cytotoxicity Assessment | 123 |
| Cell Culture | 123 |
| Sulforhodamine B Cytotoxicity Assay | 123 |
| Statistical Analysis | 124 |
| Results and Discussion | 126 |
| Microemulsion Study | 126 |
| Microemulsion Pseudo-ternary Phase Diagram | 126 |
| Characterization of Plain Microemulsions | 128 |
| Conductivity Measurement | 128 |
| Droplet Size Distribution and Zeta Potential of Plain Microemulsion | 131 |
| Evaluation of Cisplatin Loaded Microemulsion | 134 |
| Dynamic Conductivity Measurement | 134 |
| Droplet Size and Zeta Potential of Cisplatin Microemulsions | 137 |

| | |
|---|-----|
| Transmission Electron Microscopy of Cisplatin Microemulsion | 138 |
| <i>In-vitro</i> Release of Cisplatin from the Selected Microemulsion | 139 |
| Cisplatin loaded Microemulsion Based Autogel Study | 140 |
| Plain Microemulsion Based Autogel | 140 |
| Effect of Plain Microemulsion Incorporation on the Autogel Dynamic Conductivity | 141 |
| Effect of Plain Microemulsion Incorporation on Autogel Gelation Temperature | 142 |
| Cisplatin Loaded Microemulsion Based Autogel | 144 |
| Characterization of Different Cisplatin Loaded Microemulsions Based Autogel | 145 |
| pH of Cisplatin Loaded Microemulsion Based Autogel | 145 |
| Gelation Temperature and Time of Cisplatin Loaded Microemulsion Based Autogel | 145 |
| Viscosity of Cisplatin Loaded Microemulsion Based Autogel | 146 |
| Transmission Electron Microscopy of Cisplatin Loaded Microemulsion Based Autogel | 148 |
| Scanning Electron Microscopy of Cisplatin Loaded Microemulsion Based Autogel | 149 |
| <i>In-vitro</i> Drug Release from Cisplatin Loaded Microemulsion Based Autogel | 150 |
| <i>In-vitro</i> Enzymatic Degradation of Cisplatin Loaded Microemulsion Based Autogel | 152 |
| Physical Stability of Cisplatin Loaded Microemulsion Based Autogel | 154 |
| Gravitational Stability | 154 |
| Freeze Thaw Stress Test | 154 |
| Thermal Stability | 155 |
| Cytotoxicity Assessment | 163 |
| Conclusions | 168 |

***Chapter III: Biological Studies on Selected Cisplatin
Microemulsion Based Autogel***

| | |
|---|-----|
| Introduction | 171 |
| Experimental | 179 |
| Materials | 179 |
| Equipment | 180 |
| Animals | 180 |
| Methodology | 181 |
| Evaluation of <i>In-vivo</i> Gel Formation and Gel Degradation | 181 |
| <i>In-vivo</i> Cisplatin Release from Microemulsion Based Autogel | 182 |
| Acute Toxicity Evaluation | 182 |
| Experimental Design | 182 |
| Survival Rate | 183 |
| Body Weight Loss | 183 |
| Biochemical Analysis and Histopathological Examinations | 184 |
| Blood Sampling | 184 |
| Determination of Serum Creatinine | 184 |
| Determination of Serum Urea | 185 |
| Determination of Serum ALT and AST | 185 |
| Histopathological Examinations | 186 |
| Statistical Analysis | 187 |
| Results and Discussion | 188 |
| <i>In-vivo</i> Gel Formation and Degradation | 188 |
| <i>In-vivo</i> Cisplatin Release from Microemulsion Based Autogel | 192 |
| Acute Toxicity Evaluation | 195 |
| Survival Rate | 195 |
| Body Weight Loss | 197 |

| | |
|---|-----|
| Biochemical Analysis and Histopathological Examinations | 200 |
| Kidney Function Tests: Serum Creatinine and Urea | 200 |
| Liver Function Tests: Serum ALT and AST | 204 |
| Histopathological Examinations | 207 |
| Conclusions | 235 |
| General Conclusion | 238 |
| Summary | 239 |
| References | 248 |
| Arabic Summary | |
| Appendix I | |
| Appendix II | |

List of Tables

| Table No. | Title | Page |
|------------------|---|-------------|
| 1 | Gelation Temperature of Different Chitosan/ β -Glycerophosphate Thermoreversible Hydrogels | 57 |
| 2 | Effect of Dialysis on Chitosan Solution pH and Gelation Temperature of the Corresponding Thermoreversible Hydrogels | 62 |
| 3 | Composition of Dialyzed Chitosan/ β -Glycerophosphate Thermoreversible Hydrogels Selected for Cisplatin Incorporation | 64 |
| 4 | pH Values of Cisplatin Dialyzed Chitosan/ β -Glycerophosphate Hydrogels Prepared by Two Different Methods | 70 |
| 5 | Gelation Temperature of Cisplatin Dialyzed Chitosan/ β -Glycerophosphate Hydrogels Prepared by Two Different Methods | 71 |
| 6 | Gelation Time of Cisplatin Dialyzed Chitosan/ β -Glycerophosphate Hydrogels Prepared by Two Different Methods | 72 |
| 7 | Recovery of Cisplatin from Phosphate Buffer Saline (pH 7.4) | 81 |
| 8 | Intra-day Accuracy and Precision for Cisplatin in Phosphate Buffer Saline pH 7.4 | 82 |
| 9 | Inter-day Accuracy and Precision for Cisplatin in Phosphate Buffer Saline pH 7.4 | 82 |
| 10 | <i>In-vitro</i> Release Data of Cisplatin from Different Chitosan/ β -Glycerophosphate Thermoreversible Hydrogels | 84 |
| 11 | Kinetic Release Data of Cisplatin from Chitosan/ β -Glycerophosphate Thermoreversible Hydrogels | 86 |

| | | |
|----|---|-----|
| 12 | Free Cisplatin Percent as a Function to Different pH | 91 |
| 13 | <i>In-vitro</i> Release Data of Cisplatin Formula F3a with Adjusted pH | 94 |
| 14 | Composition of Plain Microemulsion Formulae and Corresponding Conductivity Values | 130 |
| 15 | Particle Size Distribution and Zeta Potential of the Selected Plain Microemulsion Formulae | 133 |
| 16 | <i>In-vitro</i> Release Data of Cisplatin from F-M (w/o) Microemulsion in Phosphate Buffer Saline (pH 7.4) | 139 |
| 17 | Effect of Plain Microemulsion Incorporation on Gelation Temperature of the Chosen Chitosan/ β -Glycerophosphate Autogel of F3 | 144 |
| 18 | <i>In-vitro</i> Release Data of Cisplatin from Microemulsion Based Autogel | 151 |
| 19 | Effect of Storage on Cisplatin Loaded Microemulsion Based Autogel Physical Properties | 156 |
| 20 | Effect of Storage at 5°C on <i>In-vitro</i> Release Data of Cisplatin from Microemulsion Based Autogel | 160 |
| 21 | Effect of Storage at 30°C on <i>In-vitro</i> Release Data of Cisplatin from Microemulsion Based Autogel | 161 |
| 22 | Weight Percent of Cisplatin Loaded Microemulsion Based Autogel Remaining at Various Time Intervals after IP Administration | 190 |
| 23 | Percentage Cisplatin Remaining in Microemulsion Based Autogel after IP Injection in Rats | 193 |
| 24 | Change in Rats Body Weight Post IP Injection of Cisplatin Solution, Cisplatin Loaded Microemulsion Based Autogel, Plain Microemulsion Based Autogel and Normal Saline | 198 |