## Preparation and evaluation of colloidal systems of an anticancer drug for transdermal delivery

A Thesis submitted

by

#### Rania Mohamed Yehia Hussain

Bachelor degree of pharmaceutical sciences, 2013, Ain Shams University

Teaching assistant, Department of pharmaceutics and Industrial pharmacy, The British University in Egypt

For the partial fulfillment of Master Degree in Pharmaceutical sciences (Pharmaceutics)

#### **Under Supervision of:**

Prof. Nahed Daoud Mortada

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

#### Prof. Dalia Abd El-Rahman Attia

Professor of pharmaceutics and Industrial pharmacy and HOD Faculty of Pharmacy, The British University in Egypt

#### Assoc. Prof. Rania Mohammed Hathout

AssociateProfessor of Pharmaceutics and Industrial pharmacy Faculty of Pharmacy, Ain Shams University

Department of pharmaceutics and Industrial pharmacy

Faculty of Pharmacy
Ain Shams University
2017

### قال تعالي:

"قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا " قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا " فَالْحَالِمُ الْحَكِيمُ" إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ"

البقرة:آية (32)

# Preparation and evaluation of colloidal systems of an anticancer drug for transdermal delivery

#### Acknowledgement

First and foremost thank to ALLAH, for helping me accomplish my thesis and work.

I would like to express my deep appreciation and sincere gratitude to **Professor Dr.**Nahed Daoud Mortada, Professor of Pharmaceutics and Industrial pharmacy,

Faculty of Pharmacy, Ain Shams University, for her valuable guidance, kindness,

helpfulness and generosity throughout the development of this work.

I would specially like to thank **Professor Dr. Dalia Abd El Rahman Attia**, HOD and Professor of Pharmaceutics and Industrial pharmacy, Faculty of Pharmacy, The British University in Egypt (BUE), for her sincerest support, supervision, assistance and motivation devoted for me and the accomplishment of this work. One could not wish for a better mentor, exemplar and extraordinary supervisor.

I offer my sincerest gratitude to Associate Professor Dr. Rania Mohamed Hathout,
Associate Professor of Pharmaceutics and Industrial pharmacy, Faculty of
Pharmacy, Ain Shams University, who taught me alot and provided me with an
amazing role model in scientific research and life. I couldn't thank her enough for
scientifically mentoring this work and her great help and efforts provided
throughout the accomplishment of this work.

I would also like to express my profound gratitude and appreciation to **Professor Dr. Mohamed Mohey ELmazar** Professor of Pharmacology and Toxicology, The

British University in Egypt (BUE), for his great help, advice, support and effort

he offered in the completion of this work. He is a true father and mentor.

<del></del>

I am also grateful for **Dr/Shaimaa Shafik Abu-Seadah**, Lecturer of Pathology, faculty of medicine (girls), Al-Azhar University for her kindness, efforts and assistance in the completion of this work.

I would also like to thank the BUE pharmacy staff for their support and Rawda Samir Mohamed; Veterinarian, Aya Adel and Moataz Sobhy Research assistances, The British University in Egypt, for their practical and technical assistance.

I would also like to thank the **members** of the department Pharmaceutics and Industrial pharmacy, Faculty of Pharmacy, The British University in Egypt (BUE), especially **Noha Alaa** for their valuable help and support.

I would also like to express my gratitude to all my friends who supported, aided and encouraged me all through the way especially **Dina Shawqy and Tasnim**El-Tantawy.

Finally, no words can describe my deepest appreciation to my mother **Professor Dr.**Hanna Shalabi, my sister Ibtesam Yehia and my brother Mostafa yehia for their aid, motivation, support and infinite patience, along with the rest of my family members for their support all through the way.

#### **Dedication**

# I dedicate this work to my beloved mother Prof. Kanna Shalabi

#### **List of contents**

Item	Page	
List of Abbreviations	I	
List of Tables	II	
List of Figures	III	
Abstract	VII	
General Introduction	1	
Scope of work	52	
Chapter I:Development, optimization and ex vivo evaluation of Methyl Dihydrojasmonate microemulsion formulations	55	
Introduction	55	
Experimental	59	
Methodology	61	
I. Ultra performance liquid chromatography (UPLC) assay of MDHJ in phosphate buffer solution (pH 7.4) and methanol (70:30, v/v).	61	
II. Construction of pseudo-ternary phase diagrams of MDHJ microemulsion systems.	64	
III. Preparation of selected microemulsion formulae according to the Simplex Lattice mixture experimental design.	65	
IV. Ex vivo permeation studies.	67	
V. Transmission Electron Microscopy (TEM)	69	

Item	Page
VI. Cytotoxicity studies	69
Results and Discussion	71
I. Ultra performance liquid chromatography (UPLC)	
assay of MDHJ in phosphate buffer solution (pH 7.4)	71
and methanol (70:30, v/v).	
II. Construction of pseudo-ternary phase diagrams of	7.6
MDHJ microemulsion systems.	76
III. Preparation of selected microemulsion formulae	
according to the Simplex Lattice mixture	81
experimental design.	
IV. Ex vivo permeation studies.	101
V. Transmission Electron Microscopy (TEM)	106
VI. Cytotoxicity studies	108
Conclusions	110
Chapter II: In vivo evaluation of an optimized MDHJ	112
microemulsion formulation.	113
Introduction	113
Experimental	116
Methodology	117
I- Animals	117
II- Induction of the solid tumors	117
III- Experimental design	118

Item	Page
IV- Determination of the solid tumor volume and the tumor inhibition rate	119
V- Statistical analysis	120
VI- Histopathological examination	120
Results and Discussion	121
I- Tumor size	121
II- Statistical analysis	124
III- The tumor inhibition rate	125
IV- Histopathological examination	127
Conclusions	135
Summary	137
References list	146
Arabic Summary	

#### **List of Abbreviations**

**Abbreviation** Meaning

ANOVA Analysis of Variance
ATP Adenosine triphosphate
AUC Area under the curve

EAC Ehrlich Ascites Carcinoma
ECS Ehrlich Solid Carcinoma

**ELISA** Rnzyme-linked immunosorbent assay

**H&E** Hematoxylin and Eosin

**HLB** Hydrophilic lipophilic balance

**HPLC** High Performance liquid chromatography

**JA** Jasmonates

MCF-7 Human breast adenocarcinoma cell line

MDHJ Methyl dihydrojasmonte

ME MicroemulsionMJ Methyl jasmonatesPDI Polydispersity index

**PS** Particle size

**ROS** Reactive oxygen species

SB Stratum basale
SC Stratum corneum
SD Standard deviation
SG Stratum granulosum
SRB Sulforhodamine B
SS Stratum spinosum

TDDS Transdermal drug delivery systems
TEM Transmission electron microscope

**UPLC** Ultra performance liquid chromatography

**VDAC** voltage-dependent anion channel

#### **List of Tables**

Table	Table Title	Page
No.	Table Title	No.
1	Summary of drug and ME emulsion systems used in literature with their corresponding transdermal fluxes and use	37
2	Different combination of 5-fluorouracil microemulsion systems with their corresponding Steady-state skin flux	46
3	Composition of the prepared MDHJ microemulsions.	65
4	Relationship between the concentration of MDHJ in phosphate buffer pH 7.4 – Methanol (70:30 v/v) and the peak areas at $\lambda$ max 190 nm using UPLC assay.	72
5	The Replicate injection of MDHJ in phosphate buffer pH 7.4 – Methanol (70:30 v/v) with their corresponding area under the curve (AUC) used for precision	74
6	The predicted MDHJ concentrations versus the determined area under the curve (AUC) with their corresponding actual concentrations and the calculated percent recovery.	75
7	Validation parameter for the developed method of analysis for MDHJ using UPLC	75
8	Droplet sizes and polydispersity indices of the Simplex lattice Mixture design points (MDHJ ME formulations).	83
9	Statistical analysis of the particle size model of the prepared ME formulation of S8 ((MDHJ:Capryol 90®) /(Labrasol®:Transcutol®)/water system).	85
10	Statistical analysis of PDI model of the prepared ME formulation of S8 ((MDHJ:Capryol 90®) / (Labrasol®/Transcutol®) / water system).	90
11	Statistical analysis of the droplet size model of the prepared ME formulation of S9 ((MDHJ : Oleic acid) / (Labrasol®/Transcutol®) / water system).	94
12	Statistical analysis of the PDI model of the prepared ME formulation of S9 ((MDHJ :Oleic acid) /(Labrasol®/Transcutol®) / water system).	98
13	MDHJ concentration, drug partitioning (KH) and diffusivity (D/H <sup>2</sup> ), together with estimated permeability coefficients (Kp) and steady state flux (Jss) of MDHJ from different microemulsion formulations	103
14	Tumor volume for the first study	122
15	Tumor volume for the second study	123

#### **List of Figures**

Figure No.	Figure Title	Page No.
1	Schematic representation of the microstructures of microemulsion.	3
2	Schematic presentation of phase diagram of microemulsion.	11
3	Schematic representation of the most of the encountered self-association structures in water, oil or a combination there.	13
4	Schematic representation of the skin.	18
5	Schematic representation of penetration routes of drugs throughout the skin.	19
6	Chemical structure of methyl dihydrojasmonate (methyl 2-(3-oxo-2-pentylcyclopentyl)acetate).	47
7	Effect of methyl jasmonate on the mitochondrial bioenergetic metabolism of cancer cells.	49
8	Methyl jasmonate mediated activities in cancer cells. MJ and other jasmonates (JAs).	51
9	Chromatogram of MDHJ in phosphate buffer pH 7.4 – Methanol (70:30 v/v), at λmax 190 nm.	71
10	Calibration curve of MDHJ in phosphate buffer pH 7.4 – Methanol (70:30 v/v) using UPLC assay at λmax 190 nm.	73
11	Pseudo ternary phase diagram for S1 MDHJ/ (Plurol Oleique®: Labrasol®)/ water system.	76
12	Pseudo ternary phase diagram for S2 MDHJ/ (Labrasol®: Transcutol®)/ water system.	77
13	Pseudo ternary phase diagram for S3 MDHJ/ (Plurol Oleique®: Tween 80®)/ water system.	77
14	Pseudo ternary phase diagram for S4 (MDHJ : Labrafac PG®)/ (Plurol Oleique®: Transcutol®)/ water.	78
15	Pseudo ternary phase diagram for S5 (MDHJ: Labrafac CC®)/ (Labrasol® : Transcutol®)/ water system.	78
16	Pseudo ternary phase diagram for S6 (MDHJ: Isopropyl myrisitate)/ (Labrasol®: Transcutol®)/water system.	79
17	Pseudo ternary phase diagram for S7 (MDHJ : Labrafil M ®)/ (Labrasol®: Transcutol®)/ water system.	79
18	Pseudo ternary phase diagram for S8 (MDHJ : Capryol 90®)/ (Labrasol® : Transcutol®)/ water system.	80

Figure No.	Figure Title	Page No.
10	Pseudo ternary phase diagram for S9 (MDHJ: Oleic acid)/	80
19	(Labrasol®: Transcutol®)/ water system.	80
	Actual versus Predicted plot for particle size (PS) of the prepared ME	
20	formulation of the systems S8 (MDHJ:Capryol 90®) /	86
	(Labrasol®/Transcutol®) / water system.	
	Box-Cox plot for Power Transforms of the prepared ME formulation	
21	of the systems S8 (MDHJ:Capryol 90®) / (Labrasol®/Transcutol®) /	87
	water system.	
	Three dimensional (3D) Simplex lattice design generated plots of the	
22	droplet size response of the prepared ME formulation of the systems	88
	S8 (MDHJ:Capryol 90®) / (Labrasol®/Transcutol®) / water system.	
	Simplex lattice generated Contour plots of the prepared ME	
23	formulation of the systems S8 (MDHJ:Capryol 90®) /	88
	(Labrasol®/Transcutol®) / water system.	
	Actual versus Predicted plot for PDI of the prepared ME formulation	
24	of the systems S8 (MDHJ:Capryol 90®) / (Labrasol®/Transcutol®) /	90
	water system.	
	Box-Cox plot for Power Transforms for PDI of the prepared ME	
25	formulation of S8 ((MDHJ:Capryol 90®) / (Labrasol®/Transcutol®) /	91
	water system).	
	Three dimensional (3D) Simplex lattice design generated plots for the	
26	PDI response of the prepared ME formulation of the systems S8	92
	(MDHJ:Capryol 90®) / (Labrasol®/Transcutol®) / water system.	
	Simplex lattice generated Contour plots plots for the PDI response of	
27	the prepared ME formulation of the systems S8 (MDHJ:Capryol 90®)	92
	/ (Labrasol®/Transcutol®) / water system.	
	Actual versus Predicted plot for particle size (PS) o of the prepared	
28	ME formulation of the systems S9 MDHJ : Oleic acid) /	94
	(Labrasol®/Transcutol®) / water.	
	Box-Cox plot for Power Transforms of the prepared ME formulation	
29	of the systems S9 MDHJ: Oleic acid) / (Labrasol®/Transcutol®) /	95
	water.	
	Three dimensional (3D) Simplex lattice design generated plots of the	
30	droplet size response of the prepared ME formulation of the systems	96
	S9 MDHJ: Oleic acid) / (Labrasol®/Transcutol®) / water.	

Figure No.	Figure Title	Page No.
31	Simplex lattice generated Contour plots of the droplet size response of the prepared ME formulation of the systems S9 MDHJ : Oleic acid) / (Labrasol®/Transcutol®) / water.	96
32	Actual versus Predicted plot for PDI of the prepared ME formulation of S9 ((MDHJ : Oleic acid) / (Labrasol®/Transcutol®) / water system).	98
33	Box-Cox plot for Power Transforms for PDI of the prepared ME formulation of S9 ((MDHJ : Oleic acid) / (Labrasol®/Transcutol®) / water system).	99
34	Simplex lattice generated Contour plots of the PDI response of the prepared ME formulation of the systems S9 MDHJ: Oleic acid) / (Labrasol®/Transcutol®) / water.	100
35	Three dimensional (3D) Simplex lattice design generated plots of the PDI response of the prepared ME formulation of the systems S9 MDHJ: Oleic acid) / (Labrasol®/Transcutol®) / water.	100
36	Cumulative amount permeated of MDHJ per unit area of the selected microemulsion formulae.	102
37	TEM images for S8 ((MDHJ:Capryol90®)/(Labrasol®:Transcutol®)/ water system)) (A x 60000 & B x 120000 ). TEM images of S9 ((MDHJ:Oleic acid)/(Labrasol®:Transcutol®) / water system)) (C x 12000 & D x 40000)	106
38	Cytotoxicity of the selected formulation compared to pure drug on MCF- 7 breast cancer cell line.	109
39	Tumor volume versus the number of doses of experimental study one.	122
40	Tumor volume versus the number of doses of the second experimental study.	123
41	The Inhibition Ratios of the first and the second study groups.	126
42	A photomicrograph of SEC sections from control mice showing sheets of small ,higher chromatophilic tumor cells of variable shape representing cell proliferation with small area of necrosis(H&E x200).	128

Figure No.	Figure Title	Page No.
43	A photomicrograph of SEC sections from control mice showing sheets of malignant cells within the muscle bundles (H&E x 400).	129
44	The photomicrographs of SEC sections from mice that received formula in experiment one; showing higher chromatophilic tumor cells with extensive necrosis (H&E x 200).	129
45	The photomicrographs of SEC sections from mice that received formula in experiment one; showing higher chromatophilic tumor cells with extensive necrosis (H&E x 400).	130
46	A photomicrograph of SEC sections from experiment two mice receiving pure drug showing sheets of high grade malignant tumor cells without necrosis (H&E x 200).	130
47	A photomicrograph of SEC sections from experiment two mice receiving Placebo showing sheets of high grade malignant tumor cells that metastasis in the muscle bundles (H&E x 200).	131
48	The photomicrographs of SEC sections from mice that received formula in experiment two; showing higher chromatophilic tumor cells with necrotic areas (H&E x 200).	131
49	The photomicrographs of SEC sections from mice that received formula in experiment two; showing higher chromatophilic tumor cells with necrotic areas (H&E x 400).	132
50	A photomicrograph of liver section (H&E x 200).	132
51	A photomicrograph of skin section showing skin tissues (H&E x 200).	133