

شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم



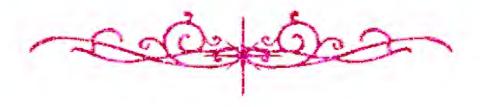
-Call 6000

سامية محمد مصطفى

شبكة المعلومات الجامعية



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم





سامية محمد مصطفى

شبكة اللفلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني واليكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



Kriver and Same

سامية محمد مصطفى

شبكة المعلومات الجامعية





سامية محمد مصطفى

شبكة اللعلومات الحامعية



بالرسالة صفحات لم ترد بالأصل





Ain shams university Faculty of pharmacy

Department of pharmaceutics and industrial pharmacy

Gelatin- based particulate systems for pulmonary delivery of an anti-cancer drug

A thesis submitted by

Hend abdelrady Mohamed abdelmohsen

Bachelor of pharmaceutical sciences, 2013, Ain shams university. Teaching assistant Ain shams university, Faculty of pharmacy, Department of pharmaceutics and industrial pharmacy.

Under supervision of

Prof. Nahed Daoud Mortada

Professor of pharmaceutics and industrial pharmacy, Faculty of pharmacy, Ain shams university.

Assoc. Prof. Rihab Osman Ahmed

Associate Professor of pharmaceutics and industrial pharmacy, Faculty of pharmacy, Ain shams university

Assoc. Prof. Rania Mohamed Hathout

Associate Professor of pharmaceutics and industrial pharmacy, Faculty of pharmacy, Ain shams university

Acknowledgements

First, I would like to thank Allah for his uncountable blessings, alhamdulillah always and forever.

I would like to express my deep appreciation to **Prof. Nahed Mortada**, the one that I have always admired since I was an undergraduate student, I used to sit in her lectures in the first line of the hall. I will always be thankful for her continous guidance, support and efforts.

I am also profoundly grateful and indebted to my supervisor dearest **Assoc. Prof. Rihab Osman**, for her continous guidance and amazingly helphul and enriching revising meetings. I'm greatly honored to work under supervision of such an inspiring researcher and a role model.

I owe my deepest gratitude to my greatest mentor, role model and sister dearest **Assoc. Prof. Rania Hathout**. There are not enough words to describe her continous support, help and motivation. She was there for me in every step through this journey from applying to the scholarship until the defense seminar. Regarding the practical work of this thesis, she was also with me from day one in the lab. I am blessed to have such a great mentor and talented person to look upto in the beginning of my career. Thanks for your constant effort, guidance and support. May Allah grant you all the happiness and success in this life.

I had to give all the credit to my mom, dad and my siblings; Nour, Basma and Mohamed, I'm literally nothing without them. Thanks for providing me with love, unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. Also, a special gratitude to my husband and best friend mahmoud. I would

especially like to thank my non blood sisters; Salma, Eman, Toka, Yasmen, Sally and all of my friends.

I also like to thank all of my colleagues in ASU and Liverpool John Moores University for their continous support and help.

Last but not least, I like to acknowledge the cultural affairs and missions sector in the ministry of higher education for giving me the opportunity to collaborate with **Prof. Imran Saleem's** laboratory under his supervision in Liverpool John Moores University.

Table of Contents

List of T	ables	l
List of fi	gures	II
List of a	bbreviations	VI
Abstract		.VIII
General	introduction	1
Scope of	work	19
Chapter	I	20
Optimiza	ation of gelatin nanoparticles fabrication process and investigation of their cellular uptake	20
Introduc	tion	20
Exper	imental	28
Metho	ods	29
1.	Preparation and optimization of gelatin nanoparticles	29
2.	Characterization of the prepared gelatin nanoparticles	30
3.	Cellular uptake of the optimized gelatin nanoparticles	31
4.	Statistical analysis	32
Result	s and discussion	33
1.	Optimized gelatin nanoparticles	33
2.	Cellular uptake of the optimized gelatin nanoparticles	42
Concl	usions	47
Chapter	II	44
Preparati	ion and characterization of methotrexate loaded gelatin nanoparticles	44
Introd	uction	49
Exper	imental	52
Metho	ods	53
1.	MTX assay using HPLC method	53
2.	MTX loading to optimized gelatin nanoparticles	54
3.	Characterization of MTX-GNPs	55
4.	In-vitro drug release	56
5.	In-vitro cytotoxicity assay	57
6.	Production of interleukine-8 (IL-8)	59
7.	Stability study	59
8.	Statistical analysis	
Result	s and discussion	
1.	Calibration curve of MTX and HPLC method validation	60

2.	MTX loading	62
3.	In-vitro drug release results	67
4.	Cytotoxicity assay	69
5.	IL-8 Production	71
6.	Stability study	73
Concl	usions	75
Chapter	III	72
•	ation of inhalable methotrexate loaded gelatin nano in-microparticles prepared by spray	72
Introd	uction	77
Exper	imental	87
Metho	ods	88
1.	Preparation of spray dried nano- in- microparticles (NIMs)	88
2.	Optimization of spray drying process using Box- Behnken design	88
3.	Characterization of spray dried gelatin nano- in- microparticles	89
4.	Statistical analysis	91
Resul	ts and discussion	92
1.	Nano- in- microparticles prepared by optimized spray drying process	92
2.	Characterization of selected spray dried MTX loaded gelatin NIMs	.100
Concl	usions	.110
General	conclusion	.112
Future p	erspectives	.113
Summar	y	.114
Referen	ces	.123
Appendi	x	

Arabic summary

List of Tables

Table	Table Title	Page
number		
1	Examples of gelatin particulate use in pulmonary drug delivery.	11
2	Process and formulation variables used in the Box-Behnken design for optimizing the gelatin NPs fabrication.	30
3	Structure of the Box- Behnken design with the corresponding experimentally determined particle size results of the prepared gelatin NPs.	34
4	Regression analysis parameters for the gelatin NPs particle size model.	36
5	Characteristics of FITC- labeled GNPs.	42
6	Median FL1-A values for control and cells treated with FITC – labeled gelatin nanoparticles.	45
7	Median SSC values for control and cells treated with FITC – labeled gelatin nanoparticles.	46
8	Validation parameters of the developed MTX HPLC assay method.	62
9	Characteristics of the fabricated gelatin NPs.	62
10	<i>In-vitro</i> release data of MTX from post loaded NPs and chemically conjugated NPs (in the presence of trypsin) in phosphate buffer pH 7.4 at 37 °C.	69
11	IC ₅₀ values for various MTX formulations on A549 cells.	71
12	Effect of storage for 30 days at 4°C on the stability of MTX-chemically conjugated and post loaded NPs.	74
13	Process variables of Box-Behnken design used for optimizing the spray drying of GNPs.	89
14	Experimentally determined yield and residual moisture content for spray dried gelatin NIMs.	93
15	Data analysis of the yield response for the spray drying technique.	94
16	Inhalation indices of spray dried NIM using next generation impactor.	108
17	In vitro deposition data of spray dried MTX-chemically conjugated NIMs determined using a next generation impactor.	109

List of figures

Figure number	Figure title	Page
I	Chemical structure of gelatin.	7
II	Schematic representation of pressurized metered dose inhaler.	14
III	Schematic illustration of (a) jet and (b) ultrasonic nebulizers.	15
IV	Schematic presentation of DPI classification according to the number of delivered doses.	16
V	Chemical structure of methotrexate.	17
VI	Schematic representation of experimental designs: (A) Box-Behnken design, (B) Central composite design, (C) Two- level full factorial design and (D) half factorial design.	25
VII	Principle of flow cytometry.	27
VIII	Cationization reaction of GNPs.	50
IX	Chemical structure of cholamine.	50
X	Performance of nanocomposite microparticles upon pulmonary delivery.	79
XI	Schematic diagram of the spray dryer.	81
XII	Co-current, counter-current and mixed flow spray dryer chambers.	82
XIII	Schematic diagram of A- spray dryer fluidized bed granulator and B- dry coating technique.	84
XIV	Examples of <i>In vitro</i> systems for pulmonary deposition investigation, A- next generation impactor, B- multistage liquid impactor and C- twin stage impinge.	85

Figure number	Figure title	Page
1	Pie chart representing the gelatin composition of amino acids.	7
2	Box-Cox plot for the PS model.	37
3	Predicted versus actual results of the PS model.	37
4	Contour plot (upper panel) and 3D surfaces (lower panel) generated by Box- Behnken design representing the effect of a- glutaraldehyde volume (GA), b-crosslinking time at-800 rpm on the particle size of the fabricated GNPs. Particle size decreases from red to blue color.	39
5	Contour plot (upper panel) and 3D surfaces (lower panel) generated by Box- Behnken design representing the effect of a- glutaraldehyde volume (GA), b-crosslinking time at 1200 rpm on the particle size of the fabricated GNPs. Particle size decreases from red to blue color.	40
6	Contour plot (upper panel) and 3D surfaces (lower panel) generated by Box- Behnken design representing the effect of a- glutaraldehyde volume (GA), b-crosslinking time at-1600 rpm on the particle size of the fabricated GNPs. Particle size decreases from red to blue color.	41
7	Effect of incubation time on the uptake of FITC- labeled GNPs into (a) macrophages and (b) A549 cells.	44
8	Fluorescence changes of (a) control macrophage cells, (b) macrophage cells treated with FITC- labeled GNPs, (c) control A549 cells and (d) A549 cells treated with FITC-labeled GNPs.	45
9	Side scattering changes of cells before and after treatment with FITC- labeled GNPs indicating the enhanced uptake into A549 cells compared to macrophage cells. (a) A549 cells treated with FITC- labeled GNPs, (b) control A549 cells, (c) macrophage cells treated with FITC- labeled GNPs and (d) control macrophage cells.	46
10	Chromatogram of MTX: mobile phase was methanol and ammonium acetate buffer (pH 6) (25:75) at 303nm.	61
11	Calibration curve of MTX constructed using methanol: ammonium acetate buffer (25:75v/v) mobile phase at 303 nm.	61
12	Effect of pH on zeta potential of plain conventional and cationized GNPs.	64
13	Effect of (a) MTX concentration and (b) stirring time on MTX loading on cationized gelatin nanoparticles.	65
14	¹ HNMR spectra for gelatin, MTX and chemically conjugated MTX and gelatin. All samples were dissolved in D2O.	66

Figure number	Figure title	Page
15	<i>In-vitro</i> release profiles for MTX post loaded NPs and MTX chemically conjugated NPs in the presence of trypsin in phosphate buffer pH 7.4.	68
16	Viabilities of A549 cells after exposure to different concentrations of plain conventional and cationized GNPs.	70
17	<i>In-vitro</i> cytotoxicity profiles for MTX solution, post loaded and chemically conjugated gelatin nanoparticles on A549 cells.	71
18	IL-8 production by A549 and macrophage cells treated with MTX, post loaded and chemically conjugated NPs. A-Control cells, B- Cells treated with MTX solution, C-Cells treated with MTX-chemically conjugated NPs and D-Cells treated with MTX-post loaded NPs.	73
19	Box-Cox plot for the yield.	95
20	Predicted versus actual results of the yield.	95
21	Contour plot (upper panel) and 3D surface (lower panel) generated by Box-Behnken design representing the effect of inlet temperature and aspirator (%) at different feed concentration at 4mg/ml on the yield (%) of spray dried NIMs. Yield (%) decrease from red to blue color.	97
22	Contour plot (upper panel) and 3D surface (lower panel) generated by Box-Behnken design representing the effect of inlet temperature and aspirator (%) at different feed concentration at 8mg/ml on the yield (%) of spray dried NIMs. Yield (%) decrease from red to blue color.	98
23	Contour plot (upper panel) and 3D surface (lower panel) generated by Box-Behnken design representing the effect of inlet temperature and aspirator (%) at different feed concentration at 12mg/ml on the yield (%) of spray dried NIMs. Yield (%) decrease from red to blue color.	99
24	TGA thermogram of NIMs.	100
25	DSC thermogram of MTX.	101
26	DSC thermogram of GNPs.	102
27	DSC thermogram of MTX- chemically conjugated GNPs.	102
28	DSC thermogram of leucine.	103
29	DSC thermogram of spray dried MTX-chemically conjugated NPs (NIMs).	103
30	XRD chart of leucine.	104
31	XRD chart of MTX.	105
32	XRD chart of spray dried MTX-chemically conjugated NPs-in-microparticles (NIMs).	105
33	SEM micrographs of spray dried MTX-chemically conjugated NPs-in-microparticles (NIMs).	106

Figure number	Figure title	Page
34	TEM images of MTX chemically conjugated NPs Abefore spray drying and B- after re-dispersion of spray dried NIMs in water.	107
35	<i>In vitro</i> deposition data of spray dried MTX-chemically conjugated NIMs determined using a next generation impactor.	109