

**OPTIMIZATION OF METHOTREXATE NANOCOMPOSITES
FORMULATIONS USING FULL FACTORIAL DESIGNS**

Prepared by

Anwar Mahmoud Hussein

Supervised by

Dr. Samer Hasan Hussein-Al-Ali

A Thesis

**Submitted to Faculty of Pharmacy as a Partial Fulfillment of the Requirements for
Master's Degree in Pharmaceutical Sciences**

Faculty of Pharmacy

Isra University

June, 2020

جامعة الاسراء

نموذج تفويض

أنا انوار محمود حسن حسين، أفوض جامعة الاسراء بتزويد نسخة من رسالتي للمكتبات أو المؤسسات أو الهيئات أو

الاشخاص عن طلبها حسب التعليمات النافذة في الجامعة.


التوقيع: 

التاريخ: 21/6/2020

Isra University

Authorization From

I'm Anwar Mahmoud Hussein, authorize Al-Isra University to supply copies of my Thesis to libraries or establishments or individuals on request, according to Al-Isra University regulations.

Signature: 

Date: 21/6/2020

COMMITTEE DECISION

This Thesis (Optimization of Methotrexate Nanocomposites Formulations Using Full Factorial Designs) was Successfully Defended and Approved on.....

Examination Committee

Signature

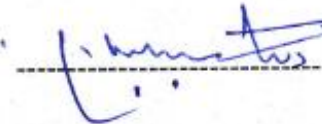
Dr. Samer Hasan Hussein-Al-Ali (Supervisor).
Associate Professor of Nanosciences.
Isra University.



Dr. Jamal Alyoussef Alkrad (Member).
Associate Professor of Pharmaceutics.
Isra University.



Dr. Dina Abdel-raouf
Professor of Pharmaceutics and Pharmaceutical Technology.
The University of Jordan.



Dedication

This project is lovingly dedicated to my sweet **Father and Mother**, whose love, affection, encouragement and whose prays of night and day support me to get such tonor and success. Without their support this research would not have made possible. Many thanks for you to teaching me to believe in God, in myself and in my dreams.

I thank God for having such caring to my parents who are the ray of my passion to science from I was young.

I would like to dedicate and express my endless thanks to **Suleiman** for his full presence and continual support.

Also to all members of my family, **my sister and my brothers**, because their care and support. I would also like to thank small kids, **Zaid and Jad**, because whose leaving my stress for long periods.

Acknowledgement

First and foremost, I would like to express my gratitude to **Dr. Samer Hussein-Al-Ali**, for awarding me the opportunity to do this project under his supervision and also for his valuable advice, teaching me some research skills, unlimited support and encouragement throughout this project work.

I am also would like to thank all my doctors whom I have learnt a lot from throughout the course of my studies.

Special thanks for **Miss Esraa Al-nsour**, **Miss FidaOdehand** for **Eng. MajdolenShawish**for their helps, support and the time I spent with them.

OPTIMIZATION OF METHOTREXATE NANOCOMPOSITES FORMULATIONS USING FULL FACTORIAL DESIGNS

ABSTRACT

In general, the importance of using nanoparticles as carriers of drug was due to high carrier capacity, high stability, probability of incorporation of both lipophilic and lipophobic drugs, and probability of various routes of administration, including inhalation route and oral route. The cyclodextrins (CDs) as drug delivery system are used to solve the problem of hydrophobicity of drugs. Therefore, methotrexate (MTX) as hydrophobic drug was incorporated into β -CD/Alg nanoparticles to form MTX- β -CD/Alg nanocomposites. The independent variables were beta-cyclodextrin, sodium alginate and calcium chloride, whereas the dependent variables were loading efficiency, Encapsulation efficiency and particle size, using full factorial design Minitab 18 software. The analysis of the model was carried out using graphical analysis such as Pareto chart, surface and contour plots, main effect plots, interaction plots, normal probability plot of the residuals and residuals versus corresponding predicted values plots. Analysis of variance (ANOVA) is structured to obtain the significant independent variables affecting the dependent response by using P value lower than 0.05. The β -CD/Alg nanoparticle samples were prepared by using different amounts of β -CD (50, 100, 200 and 500 mg) with sodium alginate at different amounts (25, 50, 100, 150 and 200 mg)

and calcium chloride (30,45,60 and 75 mg) under pH at 10 with constant mass of MTX at 50 mg. The final product of nanocomposites was separated via centrifugation at 11000 rpm for 20 min and then dried. The final nanocomposites was characterized by Fourier transform infrared(FTIR), X-ray diffraction (XRD) and *in vitro* release. FTIR test was used to evaluate the functional groups of MTX loaded β -CD/Alg nanocomposites. While XRD pattern was used to explain the interaction between MTX and its carrier (β -CD/Alg) nanoparticles. This result could be clarified by the strong interaction which destroyed the close packing for beta cyclodextrin for the formation of crystallites between methotrexate and beta cyclodextrin. After *in vitro* release study of MTX from final formulation, the results suggested that the MTX exhibited prolonged release from nanocomposites of formulation.

TABLE OF CONTENTS

ABSTRACTIV

TABLE OF CONTENTSVIII

LIST OF FIGURESXII

LIST OF TABLESXIV

LIST OF ABBREVIATIONSXV

CHAPTER 1

INTRODUCTION 1

1.1) BACK GROUND1

1.2) Problem statement 3

1.3) Design of experiments 4

1.4) Objectives 4

CHAPTER 2

LITRETURE REVIEW 5

2.1) Nanotechnology 5

2.1.1) Nano-particles and nano polymers 5

2.1.2) Nano composites polymers 6

2.1.3) Preparation of nanoparticles 7

2.2) Cyclodextrins 9

2.2.1) General properties of cyclodextrins 9

2.2.2) Classification of cyclodextrins 10

2.2.3) Complexation of cyclodextrin with drugs 11

2.2.4) Beta cyclodextrin	11	
2.2.5) complexation of drugs with β -cyclodextrin	12	
2.3) Alginate	14	
2.3.1) General properties and characterizations of alginate	14	
2.3.2) Formulation of alginate hydrogels	15	
2.3.2.1) Ionic cross-linking	15	
2.3.2.2) covalent cross-linking	16	
2.3.2.3) Thermal gelation	16	
2.3.3) complexation of drugs with alginate	16	
2.4) Chemotherapeutic drugs	18	
2.4.1) Review of formulations contain methotrexate	18	
2.5) Drug Release	20	
2.5.1) Diffusion process	20	
2.5.2) Swelling process	21	
2.5.3) Erosion process	22	
2.6) Design of experiments (DOE)	23	
CHAPTER 3		
METHODOLOGY		
3.1) Chemical materials	25	
3.2) Technical equipment	25	
3.3) Design of experiments	26	
3.4) Preparation of nanoparticles and nanocomposites		29
3.4.1) Checking the solubility		29
3.4.2) Preparation of methotrexate loaded β -cyclodextrin/alginate nanocomposites	30	

3.4.2.1) Ionotropic gelation method of alginate	30
3.4.2.2) Preparation of methotrexate/ β -cyclodextrin solution	30
3.4.2.3) Preparation of methotrexate loaded β -cyclodextrin/alginate nanocomposites	31
3.5) Instrumentation	32
3.5.1) Ultraviolet-visible spectrophotometer	32
3.5.2) Zetasizer	32
3.5.3) FTIR spectrophotometer	33
3.5.4) X-ray Diffraction	33
3.6) Loading efficiency and encapsulation efficiency of methotrexate	34
3.7) In vitro release study of methotrexate from nanocomposites	34
CHAPTER 4	
RESULT AND DISCUSSION	36
4.1) Factorial design to optimization of variables of formulation	36
4.2) Effect of formulation factors on loading efficiency, encapsulation efficiency and particle size	36
4.2.1) ANOVA values for loading efficiency, encapsulation efficiency and particle size	39
4.2.2) Residual plots for loading efficiency, encapsulation efficiency and particle size	44
4.2.3) Pareto charts of the standardized effects for loading efficiency, encapsulation efficiency and particle size	49
4.2.4) Half normal plot of the standardized effects	51
4.2.5) Contour and surface plots for loading efficiency, encapsulation efficiency and particle size	52
4.2.6) Main effect plots for loading efficiency, encapsulation efficiency and particle size	58

4.2.7) interaction plots for loading efficiency, encapsulation efficiency and particle size 60

4.3) Optimization and validation of three models 64

4.4) Fourier transform infrared (FT-IR)65

4.5) X-ray Diffraction (XRD)67

4.6) Interaction between the components of the nanocomposites 68

4.7) In Vitro release study69

CHAPTER 5

CONCLUSION 72

REFERENCES 73

APPENDIXES 81

LIST OF FIGURES

Figure 1.1	Structure of methotrexate	3
Figure 2.1	Methods of nanoparticles production	9
Figure 2.2	Types of cyclodextrin	11
Figure 2.3	Structure of beta cyclodextrin	12
Figure 2.4	Structure of sodium alginate	15
Figure 2.5	Diffusion process diagraeme	21
Figure 2.6	Swelling process diagraeme	22
Figure 2.7	Erosion process diagraeme	23
Figure 3.1	Solubility test of substances	30
Figure 3.2	Simple method of preparation methotrexate loaded B-CD/ALG	32
Figure 4.1	Normal probability plot of residuals	46
Figure 4.2	Residual versus order of data	47
Figure 4.3	Residuals versus fits of data	48
Figure 4.4	Pareto charts of the standardized effects on loading efficiency, encapsulation efficiency and particle size	50
Figure 4.5	Half normal plot for (A) loading efficiency, (B) encapsulation efficiency and (C) particle size	52
Figure 4.6	Contour and surface plots for loading efficiency against Alg, B-CD and CaCL₂ variables	53
Figure 4.7	Contour and surface plots for encapsulation efficiency against Alg, B-CD and CaCL₂ variables	55
Figure 4.8	Contour and surface plots for particle size against Alg, B-CD and CaCL₂ variables	57
Figure 4.9	Main effects plot for loading efficiency	58

Figure 4.10	Main effects plot for encapsulation efficiency	59
Figure 4.11	Main effects plot for particle size	60
Figure 4.12	Interaction plot for loading efficiency	61
Figure 4.13	Interaction plot for encapsulation efficiency	62
Figure 4.14	Interaction plot for particle size	63
Figure 4.15	The optimized concentrations for response factors of LE, EE and particle size	64
Figure 4.16	FTIR spectrum of (A) MTX, (B) β-CD, (C) Alg-βCD and (D) MTX-Alg-βCD nanocomposites	66
Figure 4.17	X-ray diffraction of (A) MTX, (B) β-CD and (C) MTX-βCD-Alg nanocomposites	67
Figure 4.18	Methotrexate loaded β-cyclodextrin/alginate nanocomposites	69
Figure 4.19	In vitro release of MTX from β-CD/Alg nanocomposites in the pH 7.4	70

LIST OF TABLES

Table 3.1	Equipment are used in experiment	25
Table 3.2	Levels for β-CD, Na-Alg and CaCL₂	26
Table 3.3	Samples by full factorial designs	27
Table 3.4	Solubility test of substances	29
Table 4.1	Data results for LE, EE and particle size	37
Table 4.2	ANOVA values for loading efficiency	39
Table 4.3	Regression equations in uncoded units for model	40
Table 4.4	Regression model for dependent variables	41
Table 4.5	ANOVA values for encapsulation efficiency	42
Table 4.6	ANOVA values for particle size	44
Table 4.7	The correlation coefficients (R²) obtained by fitting the MTX release data from β-CD/Alg nanocomposites	79

LIST OF ABBREVIATIONS

MTX	Methotrexate
B-CD	B-cyclodextrin
CaCl ₂	Calcium Chloride
CDs	Cyclodextrins
UV-vis	Ultraviolet-visible spectrophotometer
DLS	dynamic light scattering
XRD	X-Ray Diffraction
DMSO	Dimethyl Sulfoxide
CD	Cyclodextrin
SA	Sodium alginate
HCl	Hydrochloric acid
DHFR	Dihydrofolate reductase
THF	Tetrahydrofolates
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
pH	Power of hydrogen
W/V	Weight per volume
mg	Milligram
ml	Milliliter
min	Minutes
%	Percentage
°C	Celsius
IC ₅₀	Half maximal inhibitory concentration
5-FU	5-Flurouracil
M	Molarity
rpm	Rounds per minute
EE	Encapsulation efficiency
LE	Loading efficiency
FTIR	Fourier Transform Infrared Spectroscopy
λ_{\max}	Lambda max
nm	Nanometer
TGA	Thermo-gravimetric
R	Correlation of Coefficient
R ²	Coefficient of Determination