

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

Preparation and Evaluation of Medicated Intra-articular Delivery Systems

A thesis submitted in the partial fulfillment of the requirements for the Master Degree in Pharmaceutical Sciences (Drug technology)

By

May El-Zanaty Abdel Motalleb Ali Abou El-Nour

Bachelor of pharmaceutical sciences, 2011, Ain Shams University Teaching Assistant, Department of Pharmaceutics and Industrial Pharmacy Ain Shams University

Under the supervision Of

Prof. Dr. Ahmed Shawky Geneidy

Professor of Pharmaceutics and Industrial Pharmacy

Faculty of Pharmacy Ain Shams University

Dr. Mahmoud Eid Soliman

Associate professor of
Pharmaceutics and Industrial
Pharmacy
Faculty of Pharmacy
Ain Shams University

Dr. Rania Aziz Ishak

Associate professor of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy
Ain Shams University

Acknowledgment

All praise to **Allah** for enabling me to accomplish this work in its final form.

I would like to express my sincere gratefulness and deepest thanks to all my supervisors for their continuous advice, patience and valuable support throughout the development of this work.

My deepest thanks to **Prof. Dr. Ahmed Shawky Geneidy**, the professor of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, for his instructive supervision, continuous guidance and generous attitude throughout the development of this work.

I would like to express my deepest appreciation and sincere gratefulness to **Ass. Prof. Dr. Mahmoud Eid Soliman** and **Ass. Prof. Dr. Rania Aziz Ishak**, the associate professors of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, for their continuous professional guidance, instructive supervision and encouragements.

I wish to express my great appreciation and thanks to **Ass. Prof. Dr. Luca Casettari**, Associate professor at department of Biomolecular Sciences, School of Pharmacy, University of Urbino Carlo Bo, Italy, and his team for their sincere work and effort in synthesizing the copolymers used in Chapter one of this thesis.

A very special thank you to my professors, colleagues and friends in the Pharmaceutics and Industrial pharmacy department, Faculty of Pharmacy, Ain Shams University for their valuable help, support and continuous encouragement.

I would like to specially dedicate this work to my family; my dearest parents, sisters, husband and lovely daughter. None of this work would have been possible without your unconditional love, patience and continuous support. Thank you for being such a wonderful family.



List of Contents

Item	Page
List of Abbreviations	V
List of Tables	IX
List of Figures	XI
Abstract	XVI
General Introduction	1
Scope of work	30
Chapter I: Preparation and Optimization of TA-Loaded MPs	31
Introduction	31
Experimental	38
I. Materials	38
II. Equipment	38
III. Methodology	40
1. Spectrophotometric assay of TA	40
1.1. UV spectrophotometric scanning of TA in deionized water and	40
phosphate buffered saline (PBS, pH 7.4)	40
1.2. Estimation of TA calibration curves in deionized water and	40
phosphate buffered saline (PBS, pH 7.4)	40
2. Synthesis of di-block copolymer of δ-decalactone	40
3. Characterization of di-block copolymer of δ-decalactone (PEG-PDL)	41
3.1. Nuclear Magnetic Resonance (NMR)	41
3.2. Size Exclusion Chromatography (SEC)	41
4. Determination of solubility parameters	42
5. Preparation of TA-loaded MPs	43
6. Experimental design	44
6.1. Determination of drug EE% and loading efficiency (LE%)	45
6.2. Determination of PS and SI	47
7. Characterization of the optimized TA-loaded MPs	47
7.1. MPs morphology	47
7.2. Powder X-ray diffraction analysis	48
7.3. Thermal analysis	48
7.4. Fourier transform infrared (FT-IR) spectroscopy	48
8. <i>In vitro</i> release study of the optimized TA-loaded MPs	49
9. Statistical analysis	50
9.1. Data optimization using desirability function	51
Results and discussion	53
1. Spectrophotometric assay of TA	53

1.1. UV spectrophotometric scanning of TA in deionized water containing 5% methanol and PBS (pH 7.4) containing 10% methanol	53
1.2. Calibration curves of TA in deionized water and phosphate buffered	
saline (PBS, pH 7.4)	53
2. The synthesized PEG-PDL copolymer	57
3. Characterization of the synthesized PEG-PDL copolymers	57
3.1. NMR	57
3.2. SEC	60
3.3. DSC technique	61
3.4. FT-IR Spectroscopy	63
4. Solubility parameters	64
5. Preliminary studies results	68
5.1. P:D ratio factor	68
5.2. Total polymer (PLA/PEG_PDL) concentration factor	71
5.3. Type of PEG-PDL copolymer factor	71
6. Statistical analysis and modeling	74
6.1. EE% response	82
6.2. PS response	85
6.3. SI response	90
6.4. Data optimization using desirability function	92
7. Characterization of the optimized TA-loaded MPs	95
7.1. MPs morphology	95
7.2. Powder X-ray diffraction	98
7.3. Thermal analysis	101
7.4. FT-IR spectroscopy	104
8. <i>In vitro</i> drug release study	108
Conclusions	114
Chapter II: Thermo-responsive Hydrogels Loaded with Optimized TA	116
MPs	
Introduction	116
Experimental	126
I. Materials	126
II. Equipment	127
III. Methodology	128
1. Synthesis of poly(PEGMA) thermo-responsive star shaped copolymers	128
2. Characterization of the synthesized poly(PEGMA) copolymers	129
2.1. Nuclear magnetic resonance (¹ H-NMR)	129
2.2. Gel permeation chromatography (GPC)	130
2.3. Fourier transform infrared spectroscopy (FT-IR)	130

2.4. Rheometrical analysis	130
2.5. Determination of hydrophilic-lipophilic balance (HLB) and	131
hydrophilic surface areas	
2.6. Determination of cloud point by UV/visible spectroscopy	131
3. Loading the synthesized poly(PEGMA) copolymer solutions with the	131
optimized TA MPs	
4. Characterization of the prepared MPs-loaded thermo-responsive	132
hydrogels	
4.1. Determination of gelation temperature	132
4.2. Scanning electron microscope (SEM) imaging	133
4.3. Viscosity measurements	133
4.4. <i>In vitro</i> bio-adhesion study	134
4.5. In vitro TA release study	136
5. Statistical analysis	136
Results and discussion	137
1. The synthesized star-shaped thermo-responsive poly(PEGMA)	137
copolymers	
2. Characterization of the synthesized poly(PEGMA) copolymers	138
2.1. Nuclear magnetic resonance (¹ H-NMR)	138
2.2. Gel permeation chromatography (GPC)	140
2.3. Fourier transform infrared spectroscopy (FT-IR)	141
2.4. Rheometrical analysis	142
2.5. Determination of HLB and hydrophilic surface areas	145
2.6. Determination of cloud point by UV/visible spectroscopy	145
3. Characterization of the prepared MPs-loaded thermo-responsive	147
hydrogels	
3.1. Determination of gelation temperature	147
3.2. SEM imaging	151
3.3. Viscosity measurements	153
3.4. <i>In-vitro</i> bio-adhesion study	154
3.5. <i>In vitro</i> drug release study	157
Conclusions	160
Chapter III: In Vivo Evaluation of the Selected TA-Loaded Formulations	162
Introduction	162
Experimental	166
I. Materials	166
II. Equipment	166
III. Methodology	167
1. <i>In vivo</i> anti-arthritic evaluation of the selected TA-loaded formulations	167

1.1. Induction of adjuvant-induced monoarthritis (AIMA)	167
1.2. IA injection of drug treatments	168
1.3. Assessment of inflammation	169
2. Histopathological examination	170
3. Statistical analysis	171
Results and discussion	172
1. Induction of AIMA	172
2. Results for the Injection of drug treatments and assessment of	173
inflammation	
3. Histopathological examination results	185
Conclusions	199
Summary	200
References	211
Arabic Summary	1
Appendix I: Ethical Committee approval for in vivo studies	•
Appendix II: Thesis Publications	



List of Abbreviations

1,5,7-triazabicyclo[4.4.0]dec-5-ene	TBD
2,2- azobisisobutyronitrile	AIBN
Aggregates	A
Analysis of variance	ANOVA
Anti-citrullinated protein/peptide antibody	ACPA
Antigen-induced arthritis	AIA
Antigen-induced monoarthritis	AIMA
Area under the curve	AUC
Articular cartilage	AC
Biopharmaceutical classification system	BCS
Bone	В
Bone morphogenetic protein-7	BMP-7
Change in enthalpy	ΔΗ
Change in entropy	ΔS
Change in free energy of association	ΔG
Cloud point	Cpt
Coefficient of determination	R ²
Cohesive energy density	CED
Complete Freund's adjuvant	CFA
Composite injectable chitosan gels	CICGs
C-reactive protein	CRP
Critical micelle concentration	CMC
Critical micelle temperature	CMT
Cumulative percentage of drug released after 1 h	A_{1h}
Cyclooxygenase	COX
Desirability function	D
Dichloromethane	DCM
Difference between drug and polymer total solubility parameters	$\Delta \delta t$
Differential scanning calorimetry	DSC
Dimyristoyl phosphatidylethanolamine	DMPE
Dipolar parameter	$\delta_{ m p}$
Disease modifying anti-rheumatic drugs	DMARDs
Dispersion parameter	$\delta_{ m d}$
Drug-polymer interaction parameter	X_{sp}
Elastic/storage modulus	G´

Entrapment efficiency percent	EE%
Erosion	Е
Ethyl 4-aminobenzoate	EAB
Experiments and Advanced Pharmaceutical Research Unit	EAPRU
Focal necrosis	N
Fourier transform infrared spectroscopy	FT-IR
Free radical polymerization	FRP
Gel	G
Gel permeation chromatography	GPC
Glass transition temperature	$T_{\rm g}$
Granulation tissue	GT
Heat of vaporization	$\Delta H_{\rm v}$
High Resolution Field Emission Scanning Electron Microscope	HR-FESEM
Human leukocyte antigen	HLA
Hyaline cartilaginous lining	HC
Hydrogen bonding parameter	$\delta_{ m h}$
Hydrophile-lipophile balance	HLB
Incomplete Freund's adjuvant	IFA
Inflammatory cell infilterate	IF
Interleukins	IL
Intra-articular	IA
Joint space	JS
Loading efficiency percent	LE%
Loose bodies	LB
Low critical solution temperature	LCST
Major Histocompatibility complex	MHC
Matrix metalloproteinases	MMPs
Melting point temperature	Tm
Methacrylate	MA
Methotrexate	MTX
Methoxy polyethylene glycol-polypropylene fumarate	mPEG-PPF
Methoxy-polyethylene glycol-co-poly-δ-decalactone	PEG-PDL
Microparticle	MP
Microparticles	MPs
Molar volume	$V_{\rm m}$
Molecular weight	Mw
Monocyte chemoattractant protein-1	MCP-1
Monomethoxy-Polyethylene glycol	mPEG

Multinucleated osteoclasts	MNO
Nanoparticles	NPs
Non-significant	NS
Non-steroidal anti-inflammatory drugs	NSAIDs
Not applicable	NA
Not determined	ND
Nuclear Magnetic Resonance	NMR
Number-average molecular weight	Mn
One-factor-at-a-time	OFAT
Particle size	PS
Patchy chondral ossification	PO
Pentaerythritol tetra (3-mercaptopropionate)	PETMP
Phosphate buffer saline	PBS
Poly vinyl alcohol	PVA
Poly(D-Lactide)	PDLA
Poly(DL-Lactide)	PDLLA
poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene	PEO-PPO-
oxide)	PEO
Poly(L-Lactide)	PLLA
Poly(N-isopropylacrylamide)	PNIPAAm
poly(polyethylene glycol methacrylate)	poly(PEGMA)
Poly-beta amino esters	PBAEs
Poly-caprolactone	PCL
Polydispersity index	PDI
Polyester amide	PEA
Polyethylene glycol methacrylate ethyl ether	PEGMA-EE
Polyethylene glycol methacrylate methyl ether	PEGMA-ME
Poly-glycolic acid	PGA
Poly-lactic acid	PLA
Poly-lactic-co-glycolic acid	PLGA
Polymer to drug ratio	P:D
Poly-δ-decalactone	PDL
Prostaglandin E ₂	PGE_2
Receptor activator of nuclear factor-kB ligand	RANK-L
Response surface methodology	RSM
Rheumatoid arthritis	RA
Rheumatoid factor	RF
Ring opening polymerization	ROP

Scanning electron microscope	SEM
Size Exclusion Chromatography	SEC
Sodium carboxymethyl cellulose	Na CMC
Solid lipid nanoparticles	SLNs
Solubility parameter of drug	$\delta_{ m s}$
Solubility parameter of polymer	$\delta_{ m p}$
Span index	SI
Standard deviation	SD
Standard error of mean	SE
Static light scattering	SLS
Subchondral cleft	C
Subchondral cyst formation	CF
Temperature	T
Time at which 90% of drug released	$T_{90\%}$
Triamcinolone acetonide	TA
Triglycerol monostaerate	TG-18
Tumor necrosis factor-α	TNF-α
Tyramine modified hyaluronic acid	HA-Tyr
Viscous/loss modulus	G´´
Volume – weighted mean diameter	$D_{[4,3]}$
Wavelength at the maximum absorbance	$\lambda_{ ext{max}}$
Weight average molecular weight	Mw



List of Tables

Table no.	Table Title	Page
1	Different MP systems for IA drug delivery in literature.	19
2	Independent factors and their levels used for the preparation of TA-loaded MPs.	45
3	The compositions of all experimental runs for the preparation of TA-loaded MPs according to 3³-full factorial design.	46
4	Relationship between different TA concentrations in deionized water containing 5% methanol and their corresponding absorbances at $\lambda_{max} = 242$ nm.	55
5	Relationship between different TA concentrations in PBS (pH 7.4) containing 10% methanol and their corresponding absorbances at $\lambda_{max} = 242$ nm.	56
6	The Mw analysis and hydrophobic ratio of the synthesized copolymers obtained by GPC.	60
7	The Hansen's solubility parameters of TA (a), PLA (b), PDL (c), PEG (d) and PEG-PDL (e).	66
8	Partial and total solubility parameters and interaction parameter of drug and polymers.	68
9	EE%, PS and SI results of the preliminary study investigating the effect of PEG-PDL copolymer type on the characteristics of the formed MPs.	73
10	The three response results and LE% of the TA-loaded MPs prepared according to the established factorial design.	76
11	The mathematical equations for modeling the different responses.	79
12	Results of the ANOVA test for the different responses.	79
13	Predicted versus actual R ² and adequate precision of the three response models.	80
14	The absorption bands' positions of TA, PLA polymer (represented as plain MPs without copolymer), PEG-PDL ₁₇₀₀ copolymer, F-9 loaded MPs and solvent evaporated mixture.	107
15	The cumulative TA release percentages versus time data for TA suspension, F-7, F-8, and F-9 in PBS (pH 7.4) containing 10% methanol.	110