Intrapocket Bioresorbable Antimicrobial Delivery Systems for the Management of Periodontitis

A thesis submitted by

Reham Samir Atia El-Ezaby

Bachelor of Pharmaceutical Science, June 2004, Ain Shams University
Teaching assistant, Department of Pharmaceutics,
Faculty of Pharmacy, Ain Shams University

For partial fulfillment of the requirements for the Master Degree of Pharmaceutical Sciences (Pharmaceutics)

Under the Supervision
of

Prof. Dr.
Ahmed Shawky Geneidi
Professor of Pharmaceutics
and Industrial Pharmacy,
Faculty of Pharmacy,
Ain Shams University

Dr.
Gehanne Abdel Samee Awad
Associate Professor of Pharmaceutics
and Head of Department of Pharmaceutics
Faculty of Pharmacy,
Ain Shams University

Dr.
Noha Mohamed Zaki
Lecturer of Pharmaceutics,
Faculty of Pharmacy,
Ain Shams University

Ain Shams University
Faculty of Pharmacy
Department of Pharmaceutics
Cairo
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List of Abbreviations

Abbreviation	Item
BOP	Bleeding on probing
CAL	Clinical attachment level
CEJ	Cemento-enamel junction
CFUs	Colony forming units
C/GP	Chitosan/glycerophosphate
CGC	Critical gelation concentration
Ch	chitosan
CH	cholesterol
cm	centimeter
CS-HTCC/αβ-GP	quaternized chitosan/αβ-glycerophosphate
CST	Critical solution temperature
DA	Degree of acetylation
DD	Degree of deacetylation
DSC	Differntial scanning colorimetry
1DUVS	First derivative UV spectrophotometry
EVA	ethylene vinyl acetate
EPC	Egg yolk phosphatidylcholine
FT-IR	Fourier Transform Infrared Spectrometer
g	Gram(s)
GCF	Gingival crevicular fluid
GI	Gingival index
GlcNAc	N- acetyl glucosamine
GP	glycerophosphate
GVs	Giant vesicles
h	hour
HMF	5-hydroxymethylfurfural
HPLC	High performance liquid chromatography
IR	Infrared
IDDS	Intrapocket drug delivery systems
LCST	Lower critical solution temperature
LDDS	Local drug delivery system
LOVs	Large oligolamellar vesicles
LPT	Linear potentiometric titration
LUVs	Large unilamellar vesicles
MIC	Minimum inhibitory concentration
min	Minute

MLVs	Multilamellar vesicles
mm	Mellimeter
Mwt	Molecular weight
NIR	Near infrared
nm	Nanometer
NMP	N-methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
PCA	pyridine carboxylic acid
PD	Probing depth
PDI	Polydispersity index
PDLs	Periodontal ligaments
PEO	Poly ethylene oxide
PI	Plaque index
PLA	poly(lactide)
PLGA	poly(lactide-co-glycolide)
P-NIPAM	Poly(N-isopropylacrylamide)
PPZ	Poly-organophosphazenes
HBC	Hydroxy butyl chitosan
REVs	Reversed-phase evaporation vesicles
rpm	Revolution per minute
s	Second
SUVs	Small unilamellar vesicles
TEM	Transmission electron microscope
Tg	Gelation temperature
UCST	Upper critical solution temperature
USP	United States Pharmacopoeia
UV	Ultraviolet
ZCP	Zero crossing point
μm	Micrometer

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Abstract

Intrapocket Bioresorbable Antimicrobial Delivery Systems for the Management of Periodontitis

Reham Samir Atia

Department of Pharmaceutics, Faculty of Pharmacy, Ain Shams University

The aim of this thesis was to formulate ofloxacin in a novel injectable intrapocket controlled release system for the management of periodontitis. Thermoresponsive autogelling property was conferred based on the use of chitosan-β-glycerophosphate (C/β-GP) system, while more sustained release property was conferred by liposomal encapsulation of the drug. Such an approach was adopted to achieve high antibiotic concentration in the pocket, prolonged residence time, decreased side effects, decreased frequency of administration, biocompatibility, biodegradability, ease of syringeability, *in situ* gelling inside the pocket and as well as patient tolerability. Chitosan with a higher DD was prepared using the repeated alkaline hydrolysis method using NaOH 50%. Then the prepared chitosan was characterized via the determination of DD (using first derivative UV spectrophotometry in phosphoric acid) and FT-IR. C/β-GP autogels were prepared using three DD (78%, 88% and 94%), and different concentrations of glycerophosphate to modulate the gelation temperature to that of physiological range. Progressively, the prepared autogels were characterized via the determination of pH, gelation temperature, visual inspection, gelation time, mucoadhesive forces, syringeability and in vitro drug release. The results showed that chitosan with degree of deacetylation of 94% was obtained after 2h of deacetylation with NaOH

50%. Optimum preparative conditions as revealed by FT-IR analysis were: deionized water for the preparation of NaOH 50% and washing of chitosan with deionized water, methanol and acetone to decrease drying time to only 12 hrs. Determination of degree of deacetylation of chitosan by first derivative UV spectrophotometry in phosphoric acid was a valid, accurate, economic, simple and time saving method. The pH of C/β-GP depended on the concentration of β-GP and the strength of acetic acid used. Gelation temperature of C/β-GP depended on DD of chitosan and pH of the system. Mucoadhesive forces depended on DD and concentration of chitosan as well as pH of the system. Addition of ofloxacin to C/β-GP autogels increased both the pH and mucoadhesion, and decreased gelation temperature. Formulas composed of (1% chitosan with DD of 94% + 3%GP + 0.1% ofloxacin) and (2% chitosan with DD of 94% + 6%GP+ 0.1% ofloxacin) showed optimum pH (6.8), gelation temperature (36°C) and 80% drug release in three days with no erosion in the system.

Liposomes as microscopic and submicroscopic phopholipid based vesicles were used to offer more sustained antibiotic release in the periodontal pocket. Ofloxacin liposomes were prepared using the hydration and solvent evaporation methods in different phospholipid concentrations, drug concentrations and phospholipid : cholesterol ratios. Encapsulation efficiencies were determined for the prepared formulas. The formula with the best encapsulation efficiency was characterized by particle size determination, light microscope and electron microscope. Then it was incorporated into C/β-GP formulas selected from chapter I. The liposomal C/β-GP was further characterized via the determination of pH, gelation temperature, visual inspection, gelation time, mucoadhesive forces, syringeability and in vitro drug release. A maximum of 31.35%

entrapment efficiency of ofloxacin antibiotic was achieved using ofloxacin (2mg/ml), phospholipid (50mM) as well as phospholipid : cholesterol ratio (7:6). The addition of ofloxacin liposomes to C/β-GP system didn't affect pH, gelation temperature or mucoadhesive forces of the system but only decreased the gelation time. A formula composed of (1% chitosan with DD of 94% + 3%GP + 0.05% ofloxacin + liposomes equivelent to 0.05% ofloxacin) showed optimum pH (6.88), gelation temperature (34°C), syringeability and release of 80% of ofloxacin over a week. Hence, it was selected for the *in vivo* study.

The study of the *in vivo* efficacy of ofloxacin liposomal C/β-GP thermoresponsive autogel in comparison to the ordinary ofloxacin solutions was done on patients suffering from periodontitis over one week period. The study included both microbiological (on subgingival plaque samples using viable count technique) and clinical (probing depth, clinical attachment level, plaque index, gingival index and bleeding on probing) evaluation. Results revealed that ofloxacin liposomal C/β-GP thermoresponsive autogel possessed superior and prolonged antimicrobial efficiency than ofloxacin solution over seven days in contrast to ofloxacin solution which showed a rapid, but not sustained reduction in bacterial disburden. Moreover, the ofloxacin liposomal autogel showed extremely significant improvement in the five clinical parameters assessed in comparison to ofloxacin solution. Hence, ofloxacin liposomal C/β-GP thermoresponsive autogel was demonstrated to be a safe and an excellent candidate for treatment of periodontitis.

Key words: Chitosan, Glycerophosphate, Liposomes, Ofloxacin, Periodontitis, Thermoreponsive.