Mucosal Drug Delivery Systems of An Antidiabetic Drug

By

Enas Mostafa Mohamed Elmowafy

Bachelor of Pharmaceutical Science, June 2002, Ain Shams University
Master Degree in Pharmaceutics, 2008, Ain Shams University

Assistant lecturer, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University

Under the supervision of

Prof. Dr. Abdel Hameed Abdallah El-Shamy

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

Prof. Dr. Gehanne Abdel-Samie Awad

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

Dr. Rihab Osman Ahmed

Lecturer of Pharmaceutics and Industrial Pharmacy,
Faculty of Pharmacy, Ain Shams University

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

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Enas Mostafa Mohamed Elmowafy

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Dedication

I dedicate this thesis to the soul of my mother. To my dearest father, who without his help and unlimited encouragement and support in all aspects of my life, this thesis wouldn't have appeared; thank you. Words are not enough to express profound gratitude to my beloved pretty sons, who have sacrified much in these three years for whom I am forever indebted, my husband, my sister and two brothers.

Abstract

Purpose: The objective of this thesis was to develop repaglinide (REP) immediate and controlled release formulations for intranasal administration to improve the drug bioavailability and potentially overcome some of the drawbacks of the oral drug delivery.

Methods: Preparation of spray dried mucoadhesive microparticles (MPs) comprising anionic polysaccharides as well as formulation of spray dried chitosan/alginate (CS/ALG) nanocomplexes-in-microparticles (NCs-in-MPs). REP was loaded in the prepared systems.

In chapter I, the formulation parameters of REP loaded polysaccharides spray dried powders (SDP), namely, polysaccharide type (pectin (PT), gellan gum (GG) and dextran sulfate (DXT)) and drug to polymer (D/P) ratio (1:9, 1:6 and 1:3), were included in the factorial design and optimized for % release after 5 minutes ($R\%_{5min}$) and time required for 80% release ($T_{80\%}$). The suitability of the selected formulae for nasal administration was evaluated by *ex-vivo* mucoadhesion, powder insufflation and *in-vitro* cytocompatability using MTT assay.

In chapter II, the ability of two non-diabetogenic carbohydrates, namely chitosan and alginate to intranasally deliver the insulinotropic drug repaglinide (REP) for controlling blood glucose level was tested. A statistical experimental design was adopted to investigate the formulation variables effects on three critical responses: NCs size, drug entrapment efficiency (EE%) and percent REP release after 6 hours from MPs (Q_{6h}). Physicochemical characterizations of the networks structures were done and *in vitro* cytotoxicity were conducted.

In chapter III, biological investigations including pharmacodynamic study and histopathological examinations were performed on diabetic and non-diabetic Wister albino rats respectively, for the selected intranasal REP formulations.

Results: Results of chapter I showed that both polysaccharide type and amount greatly influenced the chosen responses ($R\%_{5min}$ and $T_{80\%}$). REP was highly incorporated in the mucoadhesive MPs with efficiency reaching 98.5%. The selected non-diabetogenic polysaccharides successfully improved the dissolution of REP giving 100% of drug release after 90 to 240 minutes depending on the polysaccharide used. The nasal administration of the formulae showed two to three fold increase in total blood glucose decrease compared to IN and IV solutions. The provided fast anti-hyperglycaemic effect was maintained for 6 hours. The formulae were also safe and well tolerated on nasal mucosa.

The response surface methodology used in chapter II showed that, to attain particles suitable for nasal mucosal delivery; alginate should be used at its low level (0.6 mg/mL). Low level of chitosan (0.5 mg/mL) was needed, when the drug was cation-loaded (F2), while the high chitosan level (1 mg/mL) was more suitable when REP was anion-loaded (F9). The best entrapment efficiency (EE%) was achieved at a theoretical drug loading of 0.025 mg/mL. Discrete NCs could be rapidly recovered from the spray dried MPs. The antihyperglycaemic activity of nasally administered selected NCs-in-MPs (F2 and F9) was gradual but significantly sustained over 24 hours. Nasal delivery of such dry powders achieved better glycaemic control compared to the conventional oral tablets. The cytotoxicity and histopathological studies of F2 and F9 indicated that such formulations were well tolerated.

Conclusion

Dry powder MPs were successfully prepared with particle size suitable for nasal administration. Physicochemical characteristics, morphology, drug release, swelling and mucoadhesion properties of dry powder MPs were greatly affected by anionic polysaccharide type and content. Amorphization of the drug in the three polysaccharides was proven. Gellan gum particles showed immediate release with superior swelling and

mucoadhesive properties. The pharmacodynamic study showed that formula GG2 prepared with gellan gum showed an earlier antidiabetic effect compared to REP loaded dextran sulphate and pectin formulae.

In addition, dry powder MPs containing REP loaded CS/ALG NCs were prepared and optimized in terms of NCs size, EE % and release profile using experimental design. Due to its amphoteric character, the drug ionization in the different pH values strongly affected its EE and NCs size. However, its release from the system was governed mainly by the matrix density formed. The density of the polymeric matrix and the nature of bonds affecting drug behavior in different systems were scrutinized by DSC, FT-IR and sequential TEM for recovered particles. *In vitro* and *in vivo* experiments assessed safety and tolerability of the chosen formulae.

To sum up, this study revealed that MPs and NCs-in-MPs formulations can be considered suitable carrier systems for nasal immediate and controlled delivery of REP. A proven blood glucose (BG) lowering effect in rats might suggest the potential usefulness of REP intranasal powders in the management of diabetes. The intranasal administration of the REP dry powders may help decreasing the dose and frequency of administration of the drug and possibly maximize the therapeutic benefit of the drug.

Key words: Intranasal delivery; repaglinide; microparticles; nanocomplexes; spray drying; mucoadhesion; *In vitro* cytocompatability; antidiabetic effect; histopathological examinations.

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List of Abbreviations

AFM	**Atomic force microscopy**
ALG	**Alginate**
ALG/CS	**Alginate/chitosan**
ANOVA	**Analysis of variance**
AUEC	**Area under the effect curve**
BAV	**Bioavailability**
BCS	**Biopharmaceutical classification system**
BG	**Blood glucose**
C_{min}	**Minimum glucose level as % of baseline level**
CS	**Chitosan**
conc	**Concentration**
DE	**Degree of esterification**
DXT	**Dextran sulfate**
DM	**Diabetes Millitus**
D	**Diameter of the base of the formed cone**
D%	**The total decrease in plasma glucose level**
3D	**Three dimentional**
DLS	**Dynamic light scattering**
DSC	**Differential Scanning Calorimetry**
DMSO	**Dimethyl sulfoxide**
D/P	**Drug to polymer ratio**
EE%	**Entrapment efficiency percent**
FDA	**Food and Drug Administration**
Fig	**Figure**
FITC	**Fluorescein isothiocyanate**
2FI	**Two-factor interaction**
FT-IR	**Fourier transform infrared spectroscopy**
GG	**Gellan gum**
GIP	**Gastric inhibitory polypeptide**
GLP-1	**Glucagon-like peptide-1**
GRAS	**Generally Regarded As Safe**
h	**Hour**
H	**Height of the cone**
HCl	**Hydrochloric acid**
HM	**High methoxyl**
IC_{50}	**50% inhibitory concentration**

IE%	**Incorporation efficiency percent**
IN	**Intranasal**
IR	**Infrared**
IV	**Intravenous**
kg	**Kilogram**
kV	kilovolt
K	**Procedural constant**
LEU	**Leucine**
LM	**Low methoxyl**
LMW	**Low molecular weight**
ln	**Natural logarithm**
µg	**Microgram**
µl	**Microliter**
µm	**Micrometer**
mg	**Milligram**
mL	**Milliliter**
mJ	**Millijoule**
mV	**Millivolt**
min	**Minute**
MALT	**Mucosal associated lymphoid tissue**
MEM	**Modified eagle medium**
MTT	**3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide**
MW	**Molecular weight**
MCC	**Mucociliary clearance**
MPs	**Microparticles**
nm	**Nanometer**
N	**Newton**
NaOH	**Sodium hydroxide**
NALT	**Nasal associated lymphoid tissue**
NCs	**Nanocomplexes**
ND	**Not determined**
NPs	**Nanoparticles**
NCs-in-MPs	**Nanocomplexes-in-microparticles**
PE	**Polyelectrolyte**
PEC	**Polyelectrolyte complexes**
PKa	**Ionization constant**
PS	**Particle size**
PT	**Pectin**
PBS	**phosphate buffer solution**
PI	**Polydispersity index**
Q_{6h}	**% Release after 6 hours**
R^2	**Coefficient of determination**

$R\%_{5min}$	**% Release after 5 minutes**
REP	**Repaglinide**
RSM	**Response surface methodology**
SEM	**Scanning electron microscopy**
SDP	**Spray dried powders**
SD	**Standard deviation**
SE	**Standard error**
STZ	**Streptozotocin**
SI	**Swelling index**
SS	**Sum of squares**
T_{min}	**Time point of minimum glucose level (h)**
$T_{80\%}$	**Time required for 80% release**
TEM	**Transmission Electron Microscopy**
TGA	**Thermogravimetric Analysis**
TPP	**Tripolyphosphate**
UV	**Ultraviolet**
VMD	**Volume mean diameter**
vs	**versus**
XRPD	**X-ray Powder Diffraction**
ζ	**Zeta potential**
λ_{max}	**Wavelength of maximum absorption**
ɵ	**Angle of repose**
°C	**Celsius temperature**

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