THE EFFECT OF MOLECULAR WEIGHT AND DIFFERENT DEGREE OF DEACETYLATION OF CHITOSAN ON THE IN VITRO AND IN VIVO RELEASE OF ORAL INSULIN PREPARATIONS

By

Qutuba G. Hessen

A thesis submitted in
Partial fulfillment of the
Requirements for the Degree of
Master of Science
In Pharmaceutical Sciences
at
Petra University
Faculty of pharmacy
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December 2009

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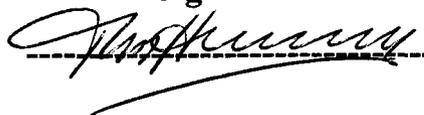## ORAL INSULIN PREPARATIONS

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<u>Supervisor</u>

Name   Signature
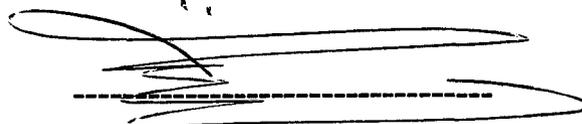
Prof. Tawfik Alhussainy

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Dr. Nidal Qinna

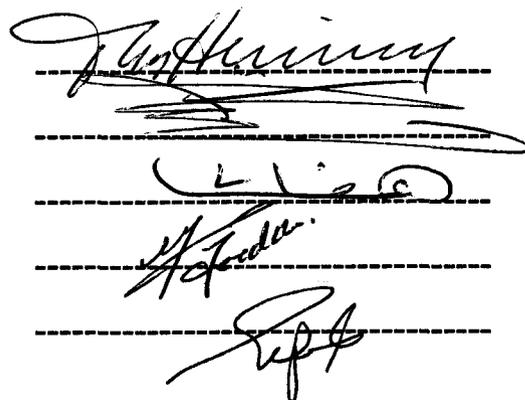<u>Examiantion Committee</u>

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Abstract

The Effect of Different Molecular Weight and Degree of Deacetylation of Chitosan On The In vitro and In vivo Release of Oral Insulin Preparation

By

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There are many barriers to delivering insulin perorally. One of the major obstacles is the harsh environment of the GIT that cause destruction of insulin. The hydrophilicity and high molecular weight of insulin represented other obstacle for its permeability across the intestinal mucosa. There are many ongoing investigations to improve the oral bioavailability of peptide and protein formulations. Chitosan has been shown to be potential in delivering oral and other mucosally administered proteins due to its excellent mucoadhesive and permeation enhancing effects across the biological surfaces. Previous studies have demonstrated the possibility of formulating an oral insulin delivery system by combining the advantages of nanoencapsulation and the use of oily vehicle. These studies revealed a necessity to well understanding the effect of molecular weight and degree of deacetylation on the formulated system. Thus, different molecular weights (1.3, 6, 13, 18, 30 kDa) and degrees of deacetylation (100, 80, 75, 65, 55 DDA%) were prepared and characterized.

In vitro studies revealed that the low molecular weight chitosan, compared to high molecular weight chitosan, had pronounced increment in its solubility due to the shortage in length of chitosan chains. This shortening of chitosan chains had its impact towards reducing the size of the formulated nanoparticles. On the other hand, and in relation to changing DDA%, the executed in silico studies explained that the flexibility of chitosan chains was increased as the DDA% decreased. At this point of

flexibility, the chitosan chains had the ability to wrapping on the insulin molecule and applying its construction power to reduce the particle size of the PEC to the nano-scale

In vivo results, using STZ diabetic rat model, demonstrated that low molecular weights of chitosan significantly enhanced the hypoglycemic effect of oral insulin. In addition; each molecular weight of chitosan had its optimum DDA%. At this DDA%, it was thought that chitosan had the best conformation to form the smallest particle size when compared to other DDA% of the same molecular weight. As a result, the lowest used molecular weight (1.3 kDa / 80%DDA) revealed to possess the best insulin release compared to the other investigated higher molecular weights.

In conclusion, the current study exposed the importance of optimizing the molecular weight of chitosan in relation with its DDA% in oral insulin delivery formulation.

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Abbreviation

DDA%	Degree of Deacetylation
Cs	Chitosan
STZ	Streptozotocin
Labrasol®	PEG-8 caprylic/capric glycerides
Plurol®	polyglyceryl-6 dioleate
IR	Infrared Spectroscopy
DSC	Differential Scanning Colarimetry
NMR	Proton Nuclear Magnetic Resonance
M.W.	Molecular Weight
min.	Minute
r.p.m.	Rotating per minute
MP	megapixel
gm	Gram
mg	Milligram
nm	Nanometer
RSD	Relative standard deviation
S.C.	Subcutaneous injection
kg	Kilogram
μmole	Micromole
M	Molarity
SEM	Scanning Electron Microscope
km	Molar ratio

Chapter One

Introduction

1. Introduction

The emerging technique of combinatorial chemistry, along with a growing knowledge of biochemistry of human body, has lead to an ever-increasing number of therapeutic proteins in the treatment of diseases. However, these proteins often lack durability that more traditional small molecule pharmaceutics possess. Where simple therapeutic agent, such as Aspirin, can be taken orally and reach the blood stream intact, the lager or more delicate protein must often be delivered directly into the bloodstream through injection. In the case of insulin, less than 0.1% of the orally dosed insulin reaches the bloodstream intact (Banting F. & Best H., 1998).

1.1. Oral delivery of protein

The oral delivery of therapeutic proteins has often been referred to as the 'Holy Grail' of drug delivery (Peppas N.A. *et al.*, 2004). Therapeutic proteins that are characterized by poor permeability across mucosal membranes commonly possess one or more of the following physicochemical characteristics: low octanol /water partitioning, presence of charged or hydrogen-bonding functional groups, and high polar surface area (Chornet E. & Dumitriu S., 1998a). However, therapeutic proteins are most frequently delivered by injection because of their large molecular weight (>3500 Da.), their hydrophilicity that prevent them to cross the lipophilic barrier of mucosal walls and the sensitive three-dimensional structure required for proteins to remain biologically active (Peppas N.A. *et*

al., 2004). Oral protein delivery is an ideal therapy because it would increase patient compliance and comfort over injection, better mimic physiologic delivery of proteins, provides a simple means of administration, reduce costs, and potentially improve the efficacy of a therapeutic treatment. Although oral protein delivery could be beneficial for many individuals, there are two key challenges to making it a successful therapy: (1) maintaining the functionality of the protein and (2) increasing the bioavailability of the drug.

Insulin, calcitonin, heparin, erythropoietin, interferon, and human growth hormone are just a few examples of therapeutic proteins actively being investigated as candidates for oral delivery. While all of those proteins are important for treating various diseases and illnesses, the focus of this research was using insulin as a model protein for oral delivery.

1.2.   Insulin and insulin therapy

Insulin is a protein composed of two polypeptide chains (5800Da.) which are covalently bound by disulfide bonds between cysteine residues (Jintapattanakit A. *et al.*, 2007a) as show in (Figure 1.1) . Insulin is secreted by β-cells of pancreatic islets in response to high blood glucose levels. Insulin is a hormone, which is necessary for glucose uptake in skeletal muscle and adipose tissue, and it also stimulates the formation of glycogen from glucose in the liver. In addition, insulin inhibits gluconeogenesis, thus slowing the hepatic glucose uptake. In contrast to insulin, glucagon is a hormone secreted by the α-cells of the pancreatic islets in response to low blood sugar. Glucagon induces the liver to secrete glucose by breaking down glycogen, thus raising the blood sugar level (Ross S.A. *et al.*, 2004).
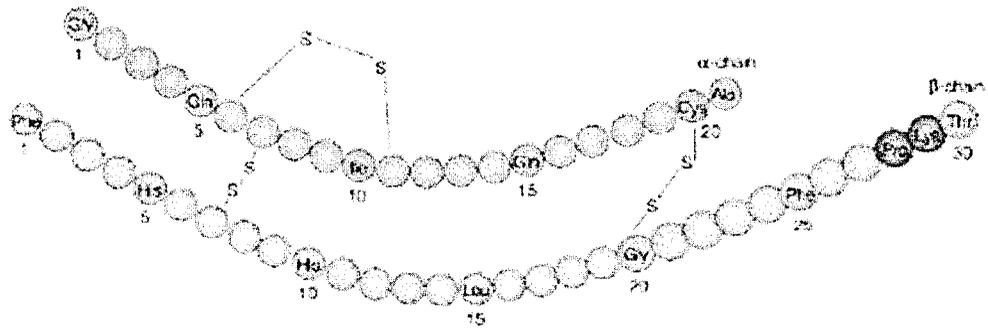
Insulin monomer

insulin dimer

insulin hexamer

Figure 1.1: Insulin molecular structure

1.3. Diabetes Mellitus

Diabetes Mellitus (DM) is a debilitating disease that is defined as the presence of high blood glucose levels due to either deficiencies in insulin production, insulin action, or both. There are approximately 150 million cases of diabetes mellitus in worldwide (Armour T.A. *et al.*, 2005). The increased glucose level can cause irreversible damage to the patient. Complications of the disease can include retinopathy, nephropathy, neuropathy, heart diseases and even death (Abolfotouh M.A., 1999).

More specifically, *Type 1 Diabetes Mellitus* (or insulin dependent diabetes mellitus, IDDM) is classified as an autoimmune destruction of the pancreatic β cells, or the insulin-producing cells of the body. The exact cause of the disease is unknown, but it thought to be induced by both genetic and environmental factors. Type 1 Diabetes is called Juvenile Diabetes as the onset of it begins in childhood (Hammami M.M., 1997a;Salsali A. & Nathan M., 2006a). *Type 2 Diabetes Mellitus* (or non-insulin dependent diabetes mellitus, NIDDM) is diagnosed when a patient begins to become resistant to insulin production and the patient may even eventually lose the ability to produce insulin. Age, obesity, family history of diabetes, race/ethnicity, and inactivity are all factors that make certain individuals more prone to developing type 2 diabetes (Yap W.S. *et al.*, 1998;Salsali A. & Nathan M., 2006b). *Gestational Diabetes (GDM)* is defined as any abnormality in glucose levels noted for the first time during pregnancy. During pregnancy, the placenta and placental hormones create an insulin resistance that is most pronounced in the last trimester. Risk assessment for diabetes is suggested starting at the first prenatal visit. High-risk individuals should be screened immediately.

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