Development of Versatile Bio-stable Oral Polymeric Delivery Systems for Proteins

By

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I, Pierre Pavan Demarco Kondiah declare that this thesis is my own work. It has being submitted for the degree of Doctor of Philosophy in the Faculty of Health Sciences in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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This ........ day of ..................... 2015
RESEARCH OUTPUTS


Research Output Presentation

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DEDICATION

“Om Namo Bhagavate Rudraya” I offer my most humble Pranams at the lotus Feet of Bhagawan Sri Sathya Sai Baba who is the source of all knowledge, wisdom and inspiration
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The end of education is character- Sri Sathya Sai Baba
Abstract

An oral proteomatrix drug delivery platform was formulated using pH responsive biostable polymers for slow release kinetics for the treatment of the neurodegenerative disease, multiple sclerosis (MS), which was the primary aim. After successful design and optimization for utilizing this system for MS, this system was further applied as a versatile platform for oral protein delivery. Interferon beta (INF-β) was selected as the oral treatment for MS. The fundamental effect of INF-β in the treatment of MS is based on reducing the immune response that is directed against central nervous system myelin, i.e. the fatty sheath that surrounds and protects nerve fibers. Damage of nerve fibers, resulting in demyelination, consequently causes nerve impulses to be slowed or halted, thus producing symptoms of MS (Jongen et al., 2011). To date, INF-β is effectively being used to treat MS subcutaneously or as intramuscular injections. These forms of administration have commonly been associated with multiple problems of pain, allergic reactions, poor patient compliance and chances of infection (Chiu et al., 2007). It was thus concluded to design an oral platform for the delivery of multiple protein therapeutic formulations. To prove the versatility of the proteomatrix system, two other demanding protein therapeutics for oral delivery, insulin and erythropoietin, were selected for further in vitro Box-Behnken series of formulations and in vivo analysis. By administration of these oral protein systems, a greater patient compliance can be achieved, thus enhancing the therapeutic profiles of patients with conditions of MS, diabetes and chronic renal failure resulting in chronic anemia. All studies consisted of in vitro drug release studies, characterization using specific analytical techniques for testing the mechanical properties, as well as the physicochemical characteristics of the copolymeric system. All proteins, INF-β, insulin and erythropoietin, were analyzed in vivo using New Zealand White rabbits (NZW) with determination of the protein from serum obtained during regular blood sampling intervals.

The polymers chitosan (CHT), trimethyl-chitosan (TMC), poly(ethylene glycol)dimethacrylate (PEGDMA) and methacrylic acid (MAA) was used in synthesis-free radical polymerization reaction, to obtain crosslinked copolymeric systems of CHT-PEGDMA-MAA and TMC-PEGDMA-MAA. The polymerization of CHT-PEGDMA-MAA produced a microgel formulation, thereby loading INF-β, insulin and erythropoietin as separate formulations for further evaluation. TMC-PEGDMA-MAA polymerization produced microparticles, loading the three proteins as separate drug delivery formulations for further in vivo and in vitro analysis. Mucoadhesive studies were undertaken on the proteomatrix systems, confirming greater mucoadhesion in the TMC crosslinked polymer than the CHT.

For insulin studies, rabbits were induced with diabetes according to the protocol approved by the university ethics committee, and evaluated for a decrease in blood glucose levels in relation to time of 24 hours. In vivo studies were undertaken comparing the oral experimental formulations, against a leading commercial product on the market for all protein formulations, administered subcutaneously, as well as compared to a control (n=3 rabbits for each group in the study). Results obtained from copolymeric TMC-PEGDMA-MAA proteomatrix microparticles concluded a greater peak absorption concentration and greater sustained release profiles for each protein formulation in vivo, as opposed to the copolymeric CHT-PEGDMA-MAA proteomatrix microgel formulation. Both TMC-PEGDMA-MAA and CHT-PEGDMA-MAA copolymeric proteomatrix formulations proved successful for all in vitro and in vivo studies to significant degrees, thus producing a versatile platform for oral protein delivery.
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LIST OF COMMONLY-USED ABBREVIATIONS

4-AP- 4-aminopyridine

AIBN- Azobisisobutyronitrile

ATR-FTIR- Attenuated Total Reflectance-Fourier Transform Infrared

BBB- Blood Brain Barrier

BET- Brunauer–Emmett–Teller

CHT- PEGDMA-MAA-Chitosan-polyethylene glycol dimethacrylate-methacrylic acid

DMT- Disease Modifying Treatments

DSC- Differential Scanning Calorimetry

EAE- Experimental autoimmune encephalomyelitis

EPO- Erythropoietin

GA- Glatiramer Acetate

INF-β- Interferon beta

MAA- Methacrylic acid

mAbs- Monoclonal antibodies

MH- Matrix hardness

MR- Matrix resilience

MS- Multiple sclerosis

$M_W$ - Molecular Weight

NMR- Nuclear Magnetic Resonance

PDI- Polydispersity Index
PEGDA- Polyethylene Glycol Diacrylate

PEGDMA- Poly(ethylene glycol) dimethacrylate

SEM- Scanning Electron Microscopy

$T_g$- Glass Transition

TGA- Thermogravimetric analysis

$T_m$- Melting Point

TMC- PEGDMA-MAA- Trimethyl chitosan polyethylene glycol dimethacrylate-methacrylic acid

XRD- X-Ray Diffraction