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A novel pH-sensitive interferon-β (INF-β) oral delivery system for application in multiple sclerosis

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ABSTRACT

pH-sensitive microparticles were prepared using trimethyl-chitosan (TMC), poly(ethylene glycol)dimethacrylate (PEGDMA) and methacrylic acid (MAA) by free radical suspension polymerization, for the oral delivery of interferon-β (INF-β). The microparticles were subsequently compressed into a suitable oral tablet formulation. A Box–Behnken experimental design was employed for generating a series of formulations with varying concentrations of TMC (0.05–0.5 g/100 mL) and percentage crosslinker (polyethylene glycol diacrylate) (3–8%, w/w of monomers), for establishment of an optimized TMC-PEGDMA-MAA copolymeric microparticles. For pragmatism, insulin was initially employed as the model peptide for undertaking the preliminary experimentation and the optimized formulation was subsequently evaluated using INF-β. The prepared copolymeric microparticulate system was characterized for its morphological, porositometric and mucoadhesive properties. The optimized microparticles with 0.5 g/100 mL TMC and 3% crosslinker had an INF-β loading efficiency of 53.25%. The *in vitro* release of INF-β was recorded at 74% and 3% in intestinal (pH 6.8) and gastric (pH 1.2) pH from the oral tablet formulation, respectively. The tablet was further evaluated for plasma concentration of INF-β in the New Zealand White rabbit, and compared to a known subcutaneous formulation. The system showed an astounding effective release profile over 24 h with higher INF-β plasma concentrations compared with the subcutaneous injection formulation.

1. Introduction

Multiple sclerosis (MS) is a chronic neurodegenerative disease that is characterized by its central nervous system effects commonly manifesting as tremors, dizziness, visual disturbances, limb weakness, muscle spasms, loss of sensation, speech impediment and depression (Fischer et al., 2011; Stuart and Bergstrom, 2011). Interferon-β (INF-β) is the most effective and widely used peptide drug for the treatment of multiple sclerosis (Jongen et al., 2011). Interferons exist naturally as a globular protein comprising of 5 helices and a molecular weight (Mw) of 20 kDa (Arduini et al., 1999). The fundamental effect of INF-β in the treatment of MS is based on reducing the immune response that is directed against myelin of the central nervous system, *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).

Presently INF-β dose is administered *via* parenteral route. But, this daily injection therapy (subcutaneously or as intramuscular injections) of INF-β has various disadvantages associated with multiple problems of pain, allergic reactions, poor patient compliance and chances of infection (Chiu et al., 2007). To overcome these problems, researchers are investigating alternative routes of delivery such as oral or pulmonary (Shaji and Patole, 2008).

The development of peptide and protein oral formulations has become an increasingly demanding form of drug delivery, and remains an attractive alternative to parenteral formulations. But there are challenges associated with the oral route of peptide delivery which include pre-systemic enzymatic degradation of the peptide and its poor permeation through the intestinal membrane (Morishita and Peppas, 2006). Absorption is the primary concern of the therapeutic in which the small intestine is the targeted area for this to occur, provided the dosage form reaches this site intact without being degraded. Translocation through the mucus layer is also an essential component, since this can severely hinder the absorption if the polymer system does not adhere to the mucus layer and release the peptide concurrently (Mustata and Dinh, 2005).

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Research in this field that aims to successfully deliver peptides orally are currently under investigation. Many strategies such as the use of enteric-coated dry emulsions, microspheres, liposomes and nanoparticles for encapsulation of peptides are being critically evaluated (Shaji and Patole, 2008). According to Veronese et al., PEGylation of proteins was investigated to have many advantages. The stability of the protein by PEGylation increases the retention time, which is one of the most beneficial advantages for absorption through the GIT. The polymer, polyethylene-glycol (PEG), shields the protein surface from degrading agents by steric hindrance in addition; the increased size of conjugate is the basis of the decreased kidney clearance of PEGylated protein (Veronese and Pasut, 2005). Studies have proved that polymers containing carboxylic acid groups have the ability to protect peptides from the protease enzymes such as trypsin and chemotrypsin. These polymers were proposed to react by binding of divalent cations (calcium and zinc) to exhibit their enzyme inhibitory effects (Sajeesh and Sharma, 2006a,b,c).

In this study pH-responsive copolymeric particles of trimethyl-chitosan-poly(ethylene glycol) dimethacrylate-methacrylic acid (TMC-PEGDMA-MAA) were successfully prepared for the oral delivery of INF-β. Using a Box–Behnken experimental design a series of formulations were synthesized and evaluated using insulin as a prototype peptide for loading and release studies, due to the high cost implications associated with extensive experimental use of INF-β. The optimized formulation was determined according to the responses from the design formulations on insulin and further evaluated for INF-β. *In vivo* studies were undertaken using New Zealand White (NZW) rabbits, comparing the developed INF-β copolymeric particulate oral delivery system with a known subcutaneous INF-β 1a product for their respective concentrations in serum during a 24-h blood sampling procedure after administration of respective formulations.

2. Materials and methods

2.1. Materials

Chitosan (CHT) (medium Mw; 450 kDa), PEG (Mw 4000 g/mol), MAA, methyl iodide, polyethylene glycol diacrylate (PEGDA), sulfonic acid, mucin (type 2) from porcine stomach and azobisisobutyronitrile (AIBN) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Humuinsulin-R (Actrapid®, r-DNA origin) of 100 IU/mL was purchased from Eli Lilly and Company (USA). INF-β 1a (Rebif®) and N-methyl-2-pyrrolidone was procured from Merck (Pty) Ltd., Modderfontein, Gauteng, South Africa at reagent grade and was used without further purification. Verikine™ Human INF-β ELISA kit was obtained from PBL Interferon Source, Piscataway, USA. All other reagents were of analytical grade and used as received. NZW rabbits weighing an average of 2.5 kg (12–15 weeks old) were provided by the Central Animal Service (CAS), University of the Witwatersrand, South Africa. The study protocol was evaluated and approved by the Research Animal Ethics Committee.

2.2. Synthesis of trimethyl-chitosan

Trimethyl-chitosan (TMC) was synthesized in a three stage procedure. In the first stage, 80 mL of N-methyl pyrrolidone was heated at 60 °C in a water bath incubating for 30 min, thereafter 2 g of CHT, 4.8 g of sodium iodide and 10 mL of 20% (w/v) sodium hydroxide solution were added. This reaction mixture was incubated in a water bath for a further 30 min at the same temperature. Methyl iodide (12 mL) was added immediately after extracting the reaction mixture from water bath and then inserted into a direct reflux apparatus using a Liebig condenser, under constant magnetic stirring of 300 rpm, for 90 min, also maintained at a temperature of 60 °C. During this phase, a thick bright yellow homogenous mixture was formed, which was precipitated using 250 mL of diethyl ether and 250 mL ethanol. The precipitate was then filtered and dried under vacuum at 60 ± 0.5 °C for 48 h. The dried polymer was finely reduced to grains and used for the next stage reaction. The second stage was the same as the first, however, no CHT was used in the reaction, instead the polymer from the first stage was used in place of the CHT. For the final stage of reaction where ion exchange of iodide ions for chloride ions occur, 80 mL of 5% (w/v) sodium chloride was prepared and the ground polymer from the previous stage was added to the solution, with continuous magnetic stirring for 30 min. The reaction mixture was then added to 250 mL of diethyl ether and 250 mL of ethanol as used previously for precipitation. After filtering the mixture, the precipitate was once again dried in the vacuum oven for 48 h at 60 °C and stored for further use.

2.3. Synthesis of PEG-dimethacrylate (PEGDMA)

High molecular weight PEG4000 and MAA were taken in a molar ratio of 1:2 for esterification reaction, using sulphonic acid as a catalyst and hydroquinine as a radical inhibitor, taken 1.5% (w/w) and 0.01% (w/w) respectively, of the monomers. The reaction was carried out in a round bottom flask, maintaining a temperature of 80–90 °C throughout in an oil bath for a period of 7 h at 40 rpm under constant magnetic stirring. Excess water was removed from the reaction using toluene, and neutralized by 5% (w/v) sodium bicarbonate solution. Ice hexane was added to precipitate the polymer PEGDMA from the solution and dried at 60 °C under vacuum conditions of 0.6 kPa for 24 h duration (Cruise et al., 1998).

2.4. Preparation of TMC-PEGDMA-MAA copolymeric particles

pH-sensitive copolymeric particles were prepared by free radical suspension polymerization technique. PEGDMA and MAA were taken in molar feed ratios of 1:2 while TMC and crosslinker PEGDA proportions were varied as 0.05–0.5 g and 3–8% (w/w) (of monomer concentration), respectively as specified in the Box–Behnken design. Free radical initiator AIBN was used as 0.6% (w/w) of monomer concentration. The polymerization reaction was carried out at controlled temperature of 75 °C, under constant purging of nitrogen gas, at 400 rpm for 6 h. The copolymeric particles thus prepared were repeatedly washed with deionized water to remove any unreacted monomers, and pH was adjusted to 7.4. The particles were finally lyophilized for further application (Tomar et al., 2011).

2.5. Application of a Box–Behnken design for copolymeric particle formulation

For designing an optimum formulation, a 2-factor, 2 level Box–Behnken experimental design was generated using Minitab® V15 statistical software (Minitab® Inc., PA, USA). The independent variables employed in the experimental design were TMC concentration (0.05–0.5 g/mL) and crosslinker amount (3–8% (w/w) of the monomer). The formulation variables were evaluated for their response on drug release in gastric (pH 1.2) and intestinal (pH 6.8) in simulated USP buffer and average particle size. Fractional release responses were evaluated at a 2-h time point in both pH mediums, with insulin taken as model peptide drug in the experimental design formulations. The design template generated 13 formulations described in Table 1. These formulations were evaluated, with the aim of acquiring high statistical scientific significance.