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Design of an *In Situ* Cross-Linked Eutectic Tablet for Enhanced Delivery of Gastro-Sensitive Proteins and Peptides

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ABSTRACT

In the present study, a eutectic platform was designed as an *in situ* cross-linked eutectic tablet for structural protection, enhanced intestinal permeation, and controlled release of proteins after oral administration. Physicochemical and physicomechanical analyses of the eutectic tablets were undertaken to elucidate the *in situ* cross-linking mechanism, thermal transitions, crystallinity, *ex vivo* permeation, and *in vitro* release of the protein. Following thermal characterization, results revealed successful eutectic formation with a melting point to 37°C. Protein release from the formulation was controlled over 24 h with a maximum fractional release of ±0.8 for all formulations. The release pattern alternated between phases of burst and slow release which was attributed to the combined effects of swelling, surface erosion, and *in situ* cross-linking. Mathematical modeling of the protein release kinetics corresponded best with the Higuichi model with near zero-order ($R^2 \approx 0.9787$) release. The permeation-enhancing effect of menthol contained within the eutectic powder blend was investigated and results showed an enhanced protein flux (0.0576-0.0720 $mg \cdot cm^{-2}\ h^{-1}$) across the intestinal tissue model compared with a control formulation. Extensive *in vitro* characterization highlighted the successful design of the eutectic tablets as a potential oral delivery system for proteins with structural protection, enhanced intestinal permeation, and controlled release.

Introduction

Oral drug delivery, particularly tablets, is considered one of the most common and widely used routes of drug administration, accounting for approximately 50% of all dosage forms on the market.[1] Tablets are considered safe and cost effective with desirable physical and chemical stability, high levels of patient acceptability, and ease of accurate dosing.[2] Despite its many advantages, oral drug delivery can be particularly challenging when considering enzymatic degradation of drug molecules within the gastrointestinal tract (GIT), low membrane permeability, and limited absorption of drugs into the systemic circulation, especially for gastro-sensitive biotech drug molecules.[3] Biotech drug molecules are unlike conventional drugs, in that they are biopharmaceutical agents such as proteins and peptides that are produced using biotechnology.[4] Owing to their complexity and inherent instability, biotech drug molecules are primarily administered parenterally (intravenously, subcutaneously, intramuscularly) as oral routes of administration may result in its degradation in the GIT.[5]

Therapeutic proteins and peptides have gained increased popularity as molecules of choice for multiple disease conditions.[6,7] Although significant progress has been made in the development of oral delivery systems for proteins and peptides, the field is limited by the GIT barrier which acts as a physical and chemical impediment in achieving successful oral drug delivery.[7-9] Pharmaceutical strategies and technologies targeting these limitations are faced with continuous challenges, preventing optimal delivery.[7] To date, this remains an active area of research.

The subject of eutectics in polymer-based protein delivery systems has not been fully explored. The microstructure that is typical of polymer eutectics still has to be explored as a strategy to increase absorption and improve the bioavailability of protein and peptide molecules.[10] A eutectic mixture is a physical blend of 2 crystalline components that are completely miscible in the liquid state but to a very limited extent in the solid state.[11] Eutectics have a melting point that is lower than that of any of the individual components contained in the mixture.[11,12] A recent study by

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Shen et al.[13] investigated the effects of a borneol/menthol eutectic mixture for enhancing the bioavailability in the delivery of a polypeptide (daidzein) used for the treatment of breast and colon cancer. The results from this study depicted the potential use of a eutectic mixture for enhancing the absorption of peptide molecules. However, the mechanism of absorption still remains unclear, reflecting no significant effect on the efflux of daidzein with the addition of a P-gp inhibitor.

Therefore, this study focused on the development of an *in situ* cross-linked eutectic tablet that provides an alternative to the parenteral route of administration for sensitive protein and peptide molecules. The premise is the design of a core eutectic region within the tablet using eutectic reagents and suitable cross-linking agents which structurally protects and ensures controlled release of the incorporated protein or peptide molecule. The structural protection is achieved by *in situ* cross-linking within the tablet core, arising from the proposed melting of the core eutectic region at body temperature (37°C) and the ingress of fluid into the tablet. In addition, the eutectic reagents within the core region of the tablet will enhance the permeation across the intestinal tissue. The outer shell of the tablet comprises a porous, fluid-absorbing polymer that maintains its characteristic shape and functions as a "sponge" that controls the release of protein or peptide molecules from the core. Using a Box–Behnken experimental design approach, a series of formulations were synthesized and analyzed using bovine serum albumin (BSA) as a prototype peptide for release and permeation studies. BSA has been widely used as a model protein in numerous studies, and thus, it was selected as a model for this study.[14-18]

Materials and Methods

Materials

Menthol (2-isopropyl-5-methylcyclohexanol, 99% purity, M_W = 15,627 g/mol), cetomacrogol 1000, poly(ethylene oxide) (PEO) (Polyox™, WSR-303), BSA (≥96%, agarose gel electrophoresis) and excipients, sodium carboxymethylcellulose (CMC), magnesium stearate, and sucrose were purchased from Sigma-Aldrich Corporation (St. Louis, MO). The cross-linker sodium carbonate ($NaCO_3$) was purchased from Associated Chemical Enterprises (Pty) Ltd. (Johannesburg, Gauteng, South Africa). Pectin citrous (poly-D-galacturonic acid methyl ester) and the cross-linker dipotassium hydrogen orthophosphate anhydrous (K_2HPO_4; M_W = 174.18 g/mol) were procured from Merck Chemicals (Pty) Ltd. (Modderfontein, Gauteng, South Africa). All other reagents were of analytical grade and were used as received.

Synthesis of the Lyophilized Eutectic Powder Blend for the Core Region to the Tablet

The lyophilized eutectic powder melt was synthesized using a co-melt method adapted from Arnikar et al.[11] Menthol and cetomacrogol 1000 were used as the eutectic reagents for formation of the eutectic melt in a 3:1 mass ratio. Menthol was initially melted at a temperature of 40 ± 0.5°C by a calibrated heated magnetic stirrer. As soon as the liquid melt was obtained, cetomacrogol 1000 was added. Once the solid mass of cetomacrogol 1000 was also melted and homogenously distributed within the molten menthol, a 5% w/w concentration of sucrose (as a cryoprotectant) was added in equal volume to the eutectic melt. After uniform distribution, the eutectic melt was then removed from the heated magnetic stirrer and allowed to cool under constant agitation at 300 rpm for ±30 minutes. The resultant eutectic blend was placed in a freezer at −80°C for 24 h before lyophilization (Labconco Freeze-Dry Systems; Labconco Corporation, Kansas City, MO) for 48 h to produce a lyophilized eutectic powder melt. Typically, lyophilization ensures the sublimation of menthol from the formulation. However, the high concentration of cryoprotectant that was added to the eutectic blend was used to protect the menthol from freezing and the associated desiccation stresses during the lyophilization process.[19-21] As a result, excess menthol was absorbed onto the cryoprotectant thereby minimizing the processing losses of menthol from the eutectic blend.

Formulation of the Core Eutectic Region for the In Situ *Cross-Linked Eutectic Tablet*

The lyophilized eutectic powder blend together with the cross-linking agents ($NaCO_3$ and K_2HPO_4) were weighed in accordance with a 3-factor Box–Behnken experimental design template (Minitab® V15 statistical software; Minitab® Inc., Philadelphia, PA) as listed in Table 1.[22] The processing parameters included the concentration of cross-linking agent, concentration of the eutectic powder blend, and the quantity of pectin. Functional components were weighed and blended with 20 mg of BSA before compression at 5 tons using a Carver Tableting Press (Wabash, IN) to produce a core eutectic tablet (5 × 2.5 mm) as illustrated in Figure 1a.

Formulation of the Outer Polymer Shell of the In Situ *Cross-Linked Eutectic Tablet*

The bottom and top layers of the outer polymer shell were prepared by measuring and blending 2 separate sets of polymeric powder blends containing 100 mg each of PEO, 12 mg of CMC, 1 mg of magnesium stearate, and specific quantities of pectin as listed in Table 1.

Assembly of the In Situ *Cross-Linked Eutectic Tablet*

A schematic of the eutectic tablet is shown in Figures 1b and 1c. The polymeric powder blend for the bottom layer of the outer polymer shell was directly compressed at 2 tons of pressure using a Carver Tableting Press loaded with punch and dies with a diameter of 10 mm to produce a loosely compressed layer. Subsequently, the core eutectic tablet was placed above this layer and centered using the tip of a needle. The polymeric powder blend for the top layer of the tablet was added above the core eutectic tablet and leveled out.

Table 1
Formulation Concentrations Generated by the Box–Behnken Design

Formulation No.	Eutectic Powder Blend (% w/w)	Cross-Linkers[a] (% w/w)	Pectin (mg)
1	12	20	80
2	12	20	65
3	18	25	65
4	18	15	65
5	18	20	72.5
6	18	20	72.5
7	12	15	72.5
8	18	25	80
9	12	25	72.5
10	24	15	72.5
11	18	20	72.5
12	24	20	80
13	24	20	65
14	24	25	72.5
15	18	15	80

[a] Cross-linker concentrations shown represent the total concentration of cross-linkers (sodium carbonate and di-potassium hydrogen orthophosphate anhydrous) added in equal parts (1:1).