Development and Evaluation of a Double-phase Multiple-unit Dosage form for Enhanced Insulin Intestinal Delivery

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Abstract: Background: The oral route is the most preferred route of administration for self-medication, but poor membrane permeability and pre-systemic degradation are key challenges that need to be addressed.

Objective: The purpose of this study was to develop and evaluate a double-phase, multiple-unit dosage form for enhanced delivery of insulin across the gastrointestinal tract epithelium. The dosage form was designed to provide increased membrane permeation by opening of tight junctions during the first phase followed by insulin delivery during the second phase.

Methods: Different beads were prepared by means of extrusion-spheronisation. Combinations of different beads constituted the double-phase drug delivery systems. The one type of bead contained insulin as active ingredient and chitosan as mucoadhesive agent, while the other bead formulations contained each one of the following drug absorption enhancers: a bile salt mixture, sodium glycocholate, Aloe vera whole leaf extract or Aloe vera gel. The insulin delivery performance of the different double-phase delivery systems was evaluated across excised pig intestinal tissues in a Sweetana-Grass diffusion apparatus.

Results: Initial exposure of the excised pig intestinal tissues to the absorption enhancer containing beads (first phase) was associated with enhanced intestinal transport of insulin (second phase) when compared to the control group. The insulin permeation enhancement effect across excised pig intestinal tissue was statistically significant in the case of pre-exposure to A. vera whole leaf extract and A. vera gel containing beads.

Conclusion: Several double-phase multiple-unit drug delivery systems have the potential to effectively deliver insulin across the gastrointestinal epithelium.

Keywords: Aloe vera, bile salt, chitosan, extrusion-spheronisation, insulin, paracellular permeation, permeation enhancement, sodium glycocholate, tight junction.

1. INTRODUCTION

Therapeutic protein and peptide drugs are predominantly administered via the parenteral route with an estimated 75% of all protein and peptide drugs currently formulated in the form of injections. Injections are associated with pain and discomfort, as well as a risk of infections and skin hardening [1]. The lack of orally administered protein and peptide drug products may be ascribed to their poor bioavailability, which is caused by poor membrane permeation (due to large molecular size and hydrophilic characteristics) as well as enzymatic degradation [2]. Despite these challenges, the use of therapeutic protein and peptide drugs is increasing due to their potential for effective treatment of a variety of diseases [1]. Therefore, a need exists for less invasive administration of protein and peptide drugs, especially for those drugs that need to be chronically administered on a regular basis (e.g. insulin). The oral route of administration presents many advantages over the parenteral route of administration such as avoiding the risk of infections and pain associated with injections [3], no need to manufacture dosage forms under sterile conditions with reduced production costs, increased patient compliance [4] and replication of the natural route of insulin secretion into the portal vein to the liver [5]. Unfortunately, the gastrointestinal tract presents physical barriers (such as the mucous layer, the biological membrane of the epithelial cells and tight junctions between the epithelium cells) and biochemical barriers (such as large pH variations and degradative enzymes) that impede effective protein and peptide...
drug delivery via the oral route [6]. Different approaches have been explored to achieve effective protein and peptide drug delivery besides exploring alternative routes other than the oral route. Of these approaches, co-administration of chemical absorption enhancers is considered a promising strategy to improve oral protein and peptide bioavailability. Chemical absorption enhancers are molecules that circumvent the physical barrier in a reversible way with low tissue injury, thus allowing the drug molecules to pass through the epithelial cells and into the systemic and lymphatic circulation [7]. Absorption enhancers are able to modulate peptide drug absorption by different mechanisms such as opening of the tight junctions, by decreasing the viscosity of the mucous layer, by improving membrane fluidity, by inhibiting efflux transporters [8, 9] and by co-administration or covalently binding to cell penetrating peptides [10]. Unfortunately, the development of effective oral delivery systems is not keeping up with the discovery and development of biotechnology-based drugs [11].

Chitosan is a biocompatible and non-toxic polymer of natural origin that can improve the paracellular permeability of peptide drug molecules across the mucosal epithelium, but also acts as a mucoadhesive agent [12-14]. Mucoadhesive properties of dosage forms provide certain benefits to protein and peptide delivery such as providing intimate contact with the mucosa and thereby protecting the drug against luminal degradation enzymes. Mucoadhesion also increases the residence time and provides a steep concentration gradient on the absorption membrane [15]. Bile salts, such as sodium glycocholate, have demonstrated the ability to increase insulin bioavailability through various mechanisms [16] such as the inhibition of protease activity, dissociation of molecular aggregates through micellar solubilisation and alteration of biological membrane integrity [17, 18]. In vivo studies in humans showed that liquid preparations containing Aloe vera (L.) Burm.f. (Aloe barbadensis Miller) gel and whole leaf extract had the ability to enhance the bioavailability of vitamins C and E [19]. Furthermore, aqueous solutions of both the whole leaf extract and gel of A. vera have been found to increase in vitro insulin transport by opening the tight junctions between intestinal epithelial cells in a reversible manner [6].

Multiple-unit drug delivery systems consist of a number of sub-units combined into a dosage form, each containing a certain portion of the total drug dose. Multiple-unit dosage forms offer several advantages over conventional single-unit drug delivery systems including a higher degree of homogeneous dispersion of the drug in the gastrointestinal tract, a reduced risk of dose dumping and a reduced risk of tissue irritation [20]. A uniform distribution of drug in small units maximizes drug absorption and reduces peak plasma level fluctuations. Furthermore, the influence of gastric emptying time as well as gastrointestinal transit time on drug absorption is minimized, which results in a more reproducible drug absorption profile with reduced intra- and inter-subject variability [21].

Several polymeric devices have been investigated for oral insulin delivery such as pH sensitive hydrogels, graft copolymer hydrogels, biodegradable polymeric devices that included polymeric micelles and polymeric nanoparticles [22-24]. A two staged single-unit drug delivery system was investigated for oral peptide drug delivery in a previous study, which consisted of an absorption enhancer and a peptide drug combined into a swellable solid oral dosage form. The development of this drug delivery system was aimed at releasing the drug absorption enhancer first to overcome the absorption barrier, followed by release of the peptide drug and subsequent absorption [25]. The purpose of this study is to develop and evaluate a double-phase multiple-unit oral dosage form that consisted of a mixture of two different types of beads (one type of bead contains insulin as well as a mucoadhesive agent and the other type of bead contains the drug absorption enhancer) for oral administration. The rationale behind the composition of this innovative type of drug delivery system was that the beads containing the drug absorption enhancer will reach the upper small intestine first and interact with the intestinal epithelium in order to increase the epithelial permeability by opening of the tight junctions (first phase). This will be followed by delivery of the insulin from the beads with increased gastrointestinal retention time due to their mucoadhesive properties at the site where enhanced absorption can take place (second phase). The insulin delivery process intended with this multiple-unit double-phase dosage form is schematically illustrated in Fig. (1).

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**Fig. (1).** Schematic illustration of the intended oral drug delivery process by the multiple-unit double-phase dosage form.
2. MATERIALS AND METHODS

2.1. Materials

*Aloe vera* gel extract powder (200:1) and chitosan were sourced from Warren Chem Pharmaceuticals (Pty) Ltd. (Kempton Park, South Africa). *A. vera* whole leaf extract was obtained from Improve USA, Inc. (DeSoto, TX, United States of America). The *A. vera* gel and whole leaf extract materials were chemically characterized by means of proton nuclear magnetic resonance spectroscopy (^1H-NMR) analysis to confirm the presence of marker molecules for *A. vera* leaf materials. Insulin, bile salt mixture (consisting of 50% sodium salt of cholic acid and 50% sodium salt of deoxycholic acid), sodium glycocholate, triethyl citrate, Krebs-Ringer Bicarbonate (KRB) buffer and sodium bicarbonate were purchased from Sigma-Aldrich (Pty) Ltd. (Johannesburg, South Africa). MicroceLac®100 (co-processed excipient consisting of lactose and microcrystalline cellulose) was obtained by Meggle (Wasserburg, Germany). The following solvents of analytical grade were purchased from Associated Chemical Enterprises (Pty) Ltd. (Johannesburg, South Africa): glacial acetic acid, acetonitrile, and hydrochloric acid. Ac-Di-Sol® (Croscarmellose Sodium) was obtained from BASF (Midrand, South Africa).

The pig proximal jejunum tissue was collected on the day of the permeation experiment from the local abattoir (Potchefstroom, South Africa). The use of animal tissues in the ex vivo permeation studies was approved by the Animal Ethics Committee at the North-West University (Potchefstroom, South Africa) (Ethics Committee approval certificate number NWU-00025-15-A5).

2.2. Bead Composition and Preparation

In order to produce a multiple-unit double-phase dosage form, two types of spherical beads (or pellets) were needed. The one type of bead formulation contained the active ingredient (i.e. insulin) together with a mucoadhesive agent (i.e. chitosan) of which only one batch was prepared. Portions of this insulin containing bead formulation were tested in combination with the other type of beads. Four other different bead formulations were prepared (one batch of each), each containing one of four selected absorption enhancers. The selected drug absorption enhancers used in this study were: *A. vera* gel, *A. vera* whole leaf extract, sodium glycocholate and a bile salt mixture (which consisted of 50% sodium salt of cholic acid and 50% sodium salt of deoxycholic acid).

For the bead formulations containing an absorption enhancer, the dry powders were weighed including MicroceLac®100 (88% w/w, filler material), Ac-Di-Sol® (2% w/w, disintegrant) and one of the selected absorption enhancers (10% w/w). The powders were blended in a Turbula® mixer (Willy A. Bachofen, Switzerland) for 5 min at 69 rpm. The total weight of each batch of bead formulation prepared was 100 g. The powder mixture of each bead formulation was wetted with an ethanol in water mixture (20% v/v) in a mortar while agitated with a pestle. The wetted powder mixtures were each passed individually through a 1 mm extrusion screen of a Type 20 Caleva® extruder (Caleva Process Solutions, England) at a speed of 25 rpm to form a spaghetti-like extrudate. This was followed by spheronisation of the extrudate using a Caleva® spheroniser (Caleva Process Solutions, England) at 1200 rpm for 6 min to form spherical beads. The beads were lyophilized by freezing them at -80°C and drying under vacuum (Virtis, Gardiner N.Y. USA) for up to 48 h. One batch of beads consisting of MicroceLac®100 only (control group for mucoadhesion) was prepared as described above.

The beads containing the active ingredient (i.e. insulin) and mucoadhesive agent (i.e. chitosan) were prepared by means of extrusion-spheronisation in a similar manner as described for the beads containing the absorption enhancers above. These beads consisted of MicroceLac®100 (82.9% w/w), insulin (0.1% w/w), chitosan (15% w/w) and Ac-Di-Sol® (2% w/w). Acetic acid (2% v/v) was added to the wetting agent and spheronisation of the extrudate was performed at 1800 rpm for 12 min.

2.3. Evaluation of the Bead Formulations

2.3.1. Insulin Content (Assay)

A sample of the insulin-containing beads (1g) was crushed using a mortar and pestle and quantitatively transferred to a volumetric flask, which was made up to 100 ml with distilled water. The insulin quantity present in the solution was determined with a validated High Performance Liquid Chromatography (HPLC) method. An HP1100 series HPLC equipped with a pump, auto-sampler, UV detector and Chemstation Rev. A.10.01 Agilent Technologies data acquisition and analysis software (Hewlett-Packard, Palo Alto, CA, USA) was used. The analysis was performed on a Vydac C18 protein and peptide column, 4.6 x 250 mm, 5 µm spherical particles, 300 Å (Grace Vydac, Hesperia, CA), using gradient elution with a mobile phase consisting of (A) HPLC grade water and 0.1% orthophosphoric acid and (B) acetonitrile, at a flow rate of 1.0 ml/min and an injection volume of 50 µl. The UV detector was set at 210 nm and the gradient conditions were set at 80% (A) for the first 6 min and then adjusted to 40% (A) and 60% (B), up to 8 min, where after it was re-equilibrated at 20% (B), and stopped at 12 min. Insulin eluted at ~5.87 min. The percentage insulin content in the bead formulation was calculated as follows:

\[
\% \text{ Insulin content} = \left( \frac{\text{Experimental value}}{\text{Theoretical value}} \right) \times 100
\]

2.3.2. Mass Variation

Ten size 0 hard gelatine capsules were manually filled by hand with each of the different bead formulations as well as combinations thereof as follows (i.e. in total 60 capsules):

- MicroceLac®100 only beads,
- Insulin and chitosan containing beads,
- Combination of insulin containing beads and *A. vera* gel containing beads,
- Combination of insulin containing beads and *A. vera* whole leaf extract containing beads,
- Combination of insulin containing beads and bile salt mixture containing beads,
• Combination of insulin containing beads and sodium glycocholate containing beads.

The mass of the contents of 10 individual capsules from each capsule formulation was compared to that of the average mass of that formulation. According to the United States Pharmacopoeia [26], capsules weighing more than 300 mg should not have more than two units with a percentage deviation of 7.5% or more from the average mass in order to comply with the specification for mass variation.

2.3.3. Friability

A sample of beads (3g) from each formulation was placed in a friability tester (TAR 220, Erweka, Heusenstamm, Germany) along with 25 glass beads (5 mm diameter). The friability tester was operated at 25 rpm for 4 min to apply 100 revolutions to each test sample. The glass beads were removed and the beads were placed on a sieve (425 µm sieve) to remove any small powder particles, before weighing the beads again. The percentage friability (%F) was determined by calculating the percentage loss in mass according to the following equation:

\[
%F = \left( \frac{\text{Initial mass} - \text{End mass}}{\text{Initial mass}} \right) \times 100
\]

2.3.4. Particle Size Analysis

The particle size distribution of the beads from each formulation was determined with laser diffraction by using a Malvern Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK), fitted with a Hydro 2000MU sample dispersion unit and a 300 mm lens. A volume of 600 ml ethanol was used as liquid dispersant for the beads and to flush the system to align apparatus optics. The Mastersizer software was used to determine the volume mean particle diameter (i.e. \(D_{4,3}\) value) and particle size distribution (i.e. \(d_{(0,5)}\) value and span).

2.3.5. Mucoadhesive Properties

The mucoadhesive properties of the bead formulations were evaluated by means of a method adapted from the “falling liquid film method” and the “adhesion number” measurement as published before [27]. The mucoadhesion of each bead formulation was tested by placing 1 g of each bead formulation onto a piece of excised pig proximal jejunum tissue that was mounted on a concave surface fitted at an angle of 15°. Distilled water (total volume of 1 l) was allowed to flow over the beads placed on the tissue, which caused some beads to wash off and accumulate in a beaker. The beads remaining on the intestine was collected and rinsed with 60 ml distilled water to remove residual mucus. The beads were then dried in a conventional oven at 40°C for 60 min and weighed. The percentage mucoadhesion was calculated using the following equation:

\[
\% \text{ Mucoadhesion} = \left( \frac{\text{Mass of beads retained on tissue}}{\text{Initial mass of beads}} \right) \times 100
\]

2.4. Excised Pig Intestinal Tissue Preparation

A portion of pig proximal jejunum tissue (± 30 cm) was collected immediately after slaughtering of the animal at the abattoir and transported in ice cold Krebs Ringer Bicarbonate (KRB) buffer in a cooler box. In the laboratory, the jejunum was pulled onto a glass rod, where after the serosa was removed with blunt dissection and the jejunum tube was cut open along the mesenteric border with a scalpel blade. The intestinal tissue was then further cut into 2 cm segments, kept moist with buffer and mounted onto the half-cells of a Sweetana-Grass diffusion apparatus [28, 29]. The half-cells were clamped together and a series of six complete chambers were placed into a heating block, each half-cell was filled with 7 ml pre-heated (37°C) KRB buffer and connected to parallel gas flow (5% CO₂; 95% O₂) at a flow rate of 15-20 ml/min. The assembled cells were equilibrated for 20 min before transepithelial electrical resistance and transport studies commenced.

2.5. Transepithelial Electrical Resistance (TEER) Studies

The effect of the different bead formulations containing drug absorption enhancers on transepithelial electrical resistance (TEER) of the excised pig intestinal tissues was determined as an indication of tight junction modulation. A Dual Channel Epithelial Voltage Clamp (Warner Instruments, Hamden, Connecticut, USA) was used to measure the initial TEER value directly before adding a sample of each of the bead formulations (0.78 g) to each of the apical chambers, followed by TEER measurements every 20 min over a period of 2 h. The percentage TEER was calculated by using the following equation:

\[
\% \text{ TEER} = \left( \frac{\text{TEER value at each time interval}}{\text{initial TEER value}} \right) \times 100
\]

2.6. Insulin Delivery Across Excised Intestinal Tissues

The delivery of insulin across excised pig intestinal tissues mounted in a Sweetana-Grass diffusion apparatus was measured after exposure to combinations of the different bead formulations. The in vitro transport study was designed in such a way to mimic the expected in vivo events after oral administration of the multiple-unit double-phase delivery systems (Fig. 1). Firstly, the mounted excised tissues were exposed to a bead formulation containing an absorption enhancer (0.78 g) for a period of 60 min (mimicking phase I), after which the beads were removed. Secondly, the mounted excised tissues were then exposed to the bead formulation containing insulin and chitosan (sufficient quantity to provide a concentration of 0.1 mg/ml insulin) for a period of 120 min (mimicking phase II). Samples (200 µl) were withdrawn from the basolateral chamber every 20 min over the 120 min period, which were replaced with pre-heated KRB buffer. The beads containing insulin and chitosan were applied to the apical chambers without pre-exposure to beads containing an absorption enhancer to determine the effect of the chitosan (included in the beads as mucoadhesive agent) on insulin transport. Insulin dissolved in KRB buffer served as a normal control for insulin transport across the excised pig intestinal tissues. The transport samples were analysed with a validated HPLC method as described above for the insulin bead assay method.

The transport results were corrected for dilution and the cumulative insulin transported was plotted as a function of time [30] to produce transport curves. The apparent permeability coefficient (\(P_{app}\)) values were calculated from the transport data using the following equation [31, 32]:

\[
P_{app} = \frac{-\ln(1-C)}{Ct} \cdot \frac{1}{L}
\]

\(C\): Concentration of insulin in the basolateral chamber
\(t\): Time
\(L\): Membrane thickness
\[ P_{\text{app}} = \frac{dQ}{dt} \left( \frac{1}{A.C_0.60} \right) \]

where \( P_{\text{app}} \) represents the apparent permeability coefficient (cm/s), \( \frac{dQ}{dt} \) is the appearance rate of the test compound at the receiver chamber over time (µg/s), \( A \) is the effective surface area of the excised tissue (cm\(^2\)) and \( C_0 \) is the initial test compound concentration in the donor chamber (µg/ml).

### 2.7. Statistical Analysis

Data analyses on the mucoadhesion, TEER and transport results were performed with STATISTICA 12 (Statsoft, Tulsa, OK, USA) with which a one-way analysis of variance (ANOVA) and Tukey’s Honest Significant Difference (HSD) post-hoc tests were performed to indicate statistically significant differences (\( p \leq 0.05 \)). All results were verified with non-parametric Kruskal-Wallis and Dunn’s post-hoc tests.

### 3. RESULTS AND DISCUSSION

#### 3.1. Insulin Content (Assay)

The insulin content of the beads prepared in this study was measured as 0.9 mg insulin per 1 g of beads. The concentration of insulin applied to the apical chamber of the Sweetana-Grass apparatus in the form of beads was determined from this measured insulin content value.

#### 3.2. Mass Variation

The average masses of the beads loaded into hard gelatine capsules as well as maximum deviations are shown in Table 1. It is clear that all the bead formulations complied with the United States Pharmacopeia [26] guidelines for mass variation.

#### 3.3. Friability

The percentage friability values of the bead formulations are shown in Table 2 and it is clear that all the bead formulations exhibited acceptable values of less than 1%. This indicates that the beads should be able to withstand mechanical stress during packaging, handling and storage.

#### 3.4. Particle Size

Table 2 also shows the particle size parameters including average particle size \([D4,3]\), median of the particle size distribution \(d(0.5)\) and span of all the bead formulations prepared in this study. The bead formulations showed relatively narrow particle size distributions, especially when the span values are considered. This indicates that the extrusion-spheronisation production technique is capable of producing relatively uniform beads, irrespective of the ingredients added.

#### 3.5. Mucoadhesive Properties

The percentage mucoadhesion values of the bead formulations are graphically presented in Fig. (2). The inclusion of chitosan in the beads resulted in a statistically significant increase (Tukey’s HSD analysis, \( p < 0.05 \)) in the mucoadhesion percentage (73.6%) when compared to that of the control group (26.2%). Chitosan was specifically added to the

### Table 1. Mass variation for the hard gelatine capsules filled with different bead formulations.

<table>
<thead>
<tr>
<th>Bead Composition</th>
<th>Average Mass (mg)</th>
<th>Max Lower (%)</th>
<th>Max Higher (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera gel (10% w/w)</td>
<td>309.1</td>
<td>7.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Aloe vera whole leaf extract (10% w/w)</td>
<td>282.4</td>
<td>6.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Sodium glycocholate (10% w/w)</td>
<td>312.8</td>
<td>5.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Bile salt mixture (10% w/w)</td>
<td>270.1</td>
<td>6.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Insulin (0.1% w/w) and Chitosan (15% w/w)</td>
<td>320.9</td>
<td>5.3</td>
<td>4.7</td>
</tr>
<tr>
<td>MicroceLac®100 only</td>
<td>275.2</td>
<td>7.2</td>
<td>3.3</td>
</tr>
</tbody>
</table>

### Table 2. Percentage friability, particle size and particle size distribution of the bead formulations.

<table>
<thead>
<tr>
<th>Bead Composition</th>
<th>Friability (%)</th>
<th>Median Particle Distribution (d(0.5)) (µm)</th>
<th>Volume Mean Diameter ([D4,3]) (µm)</th>
<th>Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera gel (10% w/w)</td>
<td>0.56 ± 0.28</td>
<td>1051.77 ± 7.27</td>
<td>1080.83 ± 7.48</td>
<td>0.65</td>
</tr>
<tr>
<td>Aloe vera whole leaf extract (10% w/w)</td>
<td>0.98 ± 0.14</td>
<td>974.29 ± 23.95</td>
<td>1005.97 ± 25.02</td>
<td>0.66</td>
</tr>
<tr>
<td>Sodium glycocholate (10% w/w)</td>
<td>0.78 ± 0.31</td>
<td>918.12 ± 7.37</td>
<td>951.25 ± 7.45</td>
<td>0.67</td>
</tr>
<tr>
<td>Bile salt mixture (10% w/w)</td>
<td>0.87 ± 0.67</td>
<td>1039.40 ± 14.24</td>
<td>1070.38 ± 13.41</td>
<td>0.67</td>
</tr>
<tr>
<td>Insulin (0.1% w/w) and Chitosan (15% w/w)</td>
<td>0.94 ± 0.28</td>
<td>841.03 ± 7.95</td>
<td>872.44 ± 8.27</td>
<td>0.67</td>
</tr>
<tr>
<td>MicroceLac®100 (100% w/w)</td>
<td>0.85 ± 0.70</td>
<td>966.71 ± 5.38</td>
<td>998.35 ± 5.02</td>
<td>0.65</td>
</tr>
</tbody>
</table>
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insulin-containing beads to increase their mucoadhesive properties, which will potentially also increase the transit time of these beads along the gastrointestinal tract. This will allow the absorption enhancer containing beads to reach the target absorption site (i.e. the upper small intestine) first in order to open the tight junctions, where after the insulin-containing beads will reach this site and deliver the insulin via the paracellular route.

Inclusion of A. vera gel as absorption enhancer in the beads caused a relatively large increase in the percentage mucoadhesion (51.7%) as compared to that of the control group (26.2%), but it was not statistically significant. The inclusion of A. vera whole leaf extract material increased the mucoadhesion to an even lower extent (34.9%). This increase in mucoadhesion for the selected aloe leaf materials may be explained by the presence of relatively large polysaccharide molecules [33] that can interact with the mucous layer on the pig intestinal tissue, resulting in increased mucoadhesion. Addition of the bile salt mixture and sodium glycocholate only caused negligible change in the mucoadhesive properties of the beads compared to that of the control group.

3.6. Transepithelial Electrical Resistance (TEER) Studies

The TEER values of the excised tissues exposed to the bead formulations containing different absorption enhancers are graphically displayed in Fig. (3) as a function of time. The control group (i.e. beads consisting of MicroceLac®100 only) showed no reduction in TEER of the excised intestinal tissues over the total 60 min exposure time. The beads containing A. vera whole leaf extract material displayed a statistically significant (Dunn’s post-hoc analysis, p < 0.05) reduction in TEER of the excised tissue over the 60 min exposure period. The beads containing A. vera gel also showed a relatively large reduction in TEER, which corresponds with the findings of Chen et al. [6] that A. vera gel is capable of...
reducing the TEER of epithelial (i.e. Caco-2) cell monolayers. A reduction in TEER suggests the opening of tight junctions between adjacent epithelial cells, which are associated with enhanced paracellular absorption of poorly absorbable drugs [34]. Beads containing bile salt mixture and sodium glycocholate also showed a reduction in TEER values, which is in accordance with a previous study [35]. Bile salts have the ability to enhance drug absorption by modulating tight junctions and changing membrane fluidity through phospholipid solubilisation [9]. From these TEER results, it is evident that the beads formulated in this study containing the different drug absorption enhancing agents have the potential to increase insulin transport across the intestinal epithelium via the paracellular route.

3.7. Insulin Delivery Across Excised Intestinal Tissues

Fig. (4) illustrates the percentage insulin transport across the excised pig intestinal tissue after exposure to combinations of the different bead formulations, while the $P_{app}$ values are shown in Table 3. No insulin could be detected in the control group (i.e. insulin alone in solution) on the basolateral side of the tissues during the entire period of 120 min, which indicated that insulin did not permeate the excised intestinal pig tissues. This was expected since peptide and protein drugs have very poor membrane permeation and usually exhibit a bioavailability of < 1% after oral administration [36]. Although insulin alone has previously been shown to be transported in vitro across intestinal epithelial membranes such as Caco-2 cell monolayers [6], it was not permeable across the excised pig intestinal tissue in this study. This can be explained by the fact that Caco-2 cell monolayers are “leakier” than intact excised animal intestinal tissues [37].

Pre-exposure of the excised pig intestinal tissues to the beads containing A. vera whole leaf extract material exhibited the highest enhancement in insulin transport (14.77%, $P_{app} = 11.64 \times 10^{-6}$ cm/s) when compared to the control group, which is in agreement with the TEER results. This constituted a statistically significant (p < 0.05) effect on the insulin transport when comparing the $P_{app}$ value of insulin after pre-exposure to the A. vera whole leaf extract beads with the $P_{app}$ value of the control group (Table 3) as determined by the Kruskal-Wallis post-hoc test. Beads containing A. vera gel also exhibited relatively high insulin transport (5.75%, $P_{app} = 3.92 \times 10^{-6}$ cm/s) when compared to that of the control group.

This enhanced insulin transport across the excised intestinal tissues can be attributed to opening of tight junctions

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**Table 3. Apparent permeability coefficient ($P_{app}$) values for insulin after pre-exposure to the various bead formulations.**

<table>
<thead>
<tr>
<th>Bead Composition</th>
<th>$P_{app}$ ($\times 10^{-6}$ cm/s)$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin solution (control)</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td><em>Aloe vera</em> whole leaf extract (10% w/w)</td>
<td>11.64 ± 1.75 ***</td>
</tr>
<tr>
<td><em>Aloe vera</em> gel (10% w/w)</td>
<td>3.92 ± 0.10 *</td>
</tr>
<tr>
<td>Sodium glycocholate (10% w/w)</td>
<td>2.74 ± 0.33 *</td>
</tr>
<tr>
<td>Bile salt mixture (10% w/w)</td>
<td>0.65 ± 0.08</td>
</tr>
<tr>
<td>Chitosan (15% w/w)</td>
<td>1.54± 0.40</td>
</tr>
</tbody>
</table>

$^*$Mean ± standard deviation of mean (n=3). * denotes a statistically significant difference from the control based on Tukey’s HSD analysis. ** denotes a statistically significant difference from the control based on a Kruskal-Wallis post-hoc test.
and thereby resulting in enhanced paracellular transport, as confirmed by the reduction in TEER and previously published results [6]. In a previous in vitro study, it was shown that both A. vera gel and whole leaf extract materials in solution were able to reduce the TEER of Caco-2 cell monolayers and enhance the transport of insulin across these monolayers [6]. In the current study, evidence was obtained that A. vera gel and whole leaf materials are capable of enhancing insulin transport across excised intestinal tissues when formulated into a solid oral dosage form. This confirmed the potential use of these A. vera leaf materials as functional excipients in solid oral dosage forms for oral insulin delivery.

The pre-exposure of excised tissues to beads containing sodium glycocholate also produced an enhancement in insulin transport (4.54%, \(P_{app} = 2.74 \times 10^{-6}\) cm/s), which is in accordance to previous findings that sodium glycocholate could enhance macromolecular drug transport [35, 38, 39]. After pre-exposure of the excised tissues to beads containing chitosan, which was included primarily as a mucoadhesive agent in this study, an insulin transport of 2.14% (\(P_{app} = 1.54 \times 10^{-6}\) cm/s) was obtained across the excised pig intestinal tissue. Although chitosan was primarily intended to act as a mucoadhesive agent in the double-phase delivery system developed in this study, it was also capable of opening tight junctions which mediated an increase in the paracellular permeability of peptide drugs as was previously shown. The relatively low increase in insulin transport caused by chitosan was probably due to the neutral pH of the KRB buffer where chitosan is poorly soluble [12, 40].

This in vitro transport study provided proof of concept that the double-phase multiple-unit pellet systems are capable of delivering insulin across the intestinal epithelium when the epithelium is first exposed to beads containing an absorption enhancer and then exposed to beads containing the insulin. However, no claims regarding therapeutic efficacy can be made based on in vitro studies only and in vivo studies are needed to determine the extent of bioavailability enhancement that can be achieved by this double-phase dosage form.

**CONCLUSION**

All the drug absorption enhancing agents incorporated into beads resulted in TEER reduction of excised pig intestinal tissue, indicating the ability to open tight junctions between adjacent epithelial cells. The beads containing the A. vera whole leaf extract material exhibited the highest TEER reduction and, in accordance with this result, also achieved the highest insulin delivery across excised pig intestinal tissue. This study showed, by means of in vitro testing, that several double-phase multiple-unit drug delivery systems have potential for effective delivery of insulin across the gastrointestinal epithelium. However, follow-up in vivo studies are necessary to confirm the efficacy of this double-phase delivery system in terms of providing therapeutic levels of peptide drugs via the oral route of drug administration.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Although no animals were used in this study, ethics approval was obtained from the North-West University Research Ethics Regulatory Committee for use of pig intestinal tissues obtained from an abattoir (ethics approval number: NWU-00025-15-A5) for the in vitro permeation and muco-adhesion studies.

**HUMAN AND ANIMAL RIGHTS**

No Animals/Humans were used for studies that are base of this research.

**CONSENT FOR PUBLICATION**

Not applicable.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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