Chitosan-based lyophilised xerogels for potential buccal delivery of macromolecules.

I. Ayensu, H. Pawar, J. C. Mitchell, J.S. Boateng
Overview

• Background

• Formulation design and optimisation

• Physico-chemical/bio-analytical characterisation

• Stability evaluation

• Permeation studies

• Summary and conclusions
Background – Mucosal Drug Delivery

Rational

• Challenges associated with traditional routes
  – Enzymatic breakdown in GIT and hepatic first pass effect.
  – Injections require sterility, expensive and pain results in patient non-compliance.

• Special patient groups
  – Children
  – Ageing population
  – Difficulty in swallowing

• New markets (patent expiration)
Buccal mucosa drug delivery

Pros

• Large surface area for absorption (50.2 cm²)
• Minimal peptidase activities
• Richly vascularised
• Avoids first pass metabolism, GIT degradation and pain
• Easy removal of dosage form in case of irritation
• Increased patient compliance

Cons

• Drug molecular size
• Hydrophobicity
• Low membrane permeability

Improving protein absorption via the buccal mucosa

• The use of absorption enhancers
• Enzyme inhibitors
• Muco-adhesive systems

Bioadhesive polymers

Typical polymers
- Carbopol, polycarbophil
- Polyethylene oxide
- Carboxymethylcellulose
- Sodium alginate, xanthan,
- Chitosan and derivatives

Well characterised for safety and compatibility
- Able to deliver hydrophilic drugs
- Bioadhesive

Ayensu et al. 2012  Carbohydrate Polymers
Formulation design and optimisation

**TG-chitosan xerogel**

- **EDAC 50mM**
- **Thioglycolic acid 500mg**
- **Chitosan (500mg) + 0.1M HCl**
- **pH 5, 1M NaOH**
- **Chitosan-TGA Stirred at room temp. for 3-4hrs**
- **Membrane dialysis (in aqueous media)**
- **Annealed**
- **Lyophilization**
- **Non-annealed**

**Physico-chemical characterisation**

<table>
<thead>
<tr>
<th></th>
<th>Chitosan</th>
<th>TG-chitosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel permeation chromatography (GPC)</td>
<td>196,744 271 Da</td>
<td>200,708 Da</td>
</tr>
<tr>
<td>Ellman’s reaction</td>
<td>-</td>
<td>236 ± 26 μmol thiol/g polymer.</td>
</tr>
<tr>
<td>ATR-FT-IR</td>
<td>[a] chitosan</td>
<td>[b] TG-chitosan</td>
</tr>
</tbody>
</table>

 Ayensu et al. 2012 Carbohydrate Polymers
## Formulations

<table>
<thead>
<tr>
<th>Component</th>
<th>Formulations (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Formulation A</td>
</tr>
<tr>
<td>TG-chitosan</td>
<td>100</td>
</tr>
<tr>
<td>Mannitol</td>
<td>10</td>
</tr>
<tr>
<td>Glycerol</td>
<td>10</td>
</tr>
<tr>
<td>Brij 35</td>
<td>10</td>
</tr>
<tr>
<td>Glutathione</td>
<td>0</td>
</tr>
<tr>
<td>BSA</td>
<td>50</td>
</tr>
</tbody>
</table>

### Preparation of EC laminated chitosan/TG-chitosan xerogels.

![Diagram of EC laminated chitosan/TG-chitosan xerogels preparation process]
EC film as backing membrane for xerogels (x200 – x10,000).

SEM image of Xerogel (A) on EC backing membrane (B)
## Hydration capacity

<table>
<thead>
<tr>
<th>Protein</th>
<th>Annealing Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG-chitosan-BSA</td>
<td>Annealed without DTT</td>
<td>480.01 ± 18.16</td>
</tr>
<tr>
<td>TG-chitosan-BSA</td>
<td>Annealed with DTT</td>
<td>1086.80 ± 5.10</td>
</tr>
<tr>
<td>TG-chitosan-GSH-BSA</td>
<td>Annealed without DTT</td>
<td>912.50 ± 25.08</td>
</tr>
<tr>
<td>TG-chitosan-GHS-BSA</td>
<td>Annealed with DTT</td>
<td>910.24 ± 24.70</td>
</tr>
</tbody>
</table>
Mucoadhesion

Effect of mucin concentration

Effect of GSH concentration

Effect of enzyme inhibitors on drug release

*In-vitro* drug release in 0.01M PBS (pH 6.8) using franz-type diffusion cell at 37 °C.
Stability studies

**Circular dichroism**

![Circular dichroism graph](image)

Wavelength (nm)

- Chitosan-BSA
- TG-chitosan-BSA
- BSA
- TG-chitosan-GSH-BSA

**ATR-FT-IR**

![ATR-FT-IR graph](image)

Wave number (cm\(^{-1}\))

- BSA
- TG-chitosan-BSA
- TG-chitosan-BSA-GSH

Transmittance

- Amide I
- Amide II
Permeation studies (insulin)

**Sheep**

- **EpiOral™**
  - Cumulative INS permeated (µg/cm²) over time (hours)
  - R² = 0.9124

- **Sheep**
  - Cumulative INS permeated (mg/cm²) over time (hours)
  - R² = 0.9953

**EpiOral™**

- Cumulative INS permeated (µg/cm²) over time (hours)

**TG-chitosan-INS permeation**

- **Sheep**
  - TG-chitosan-INS permeation (Epi-oral)
  - R² = 0.9124

- **Sheep**
  - TG-chitosan-INS permeation (Epi-oral) vs. TG-chitosan-INS permeation (Sheep)
Summary conclusions

• Optimised chitosan based xerogels have been produced and characterised.

• Annealing, thiolation and enzyme inhibitors significantly affect functional characteristics such as hydration, mucoadhesion, \textit{in vitro} drug release and permeation.

• The xerogels showed low toxicity with conformationally stable model protein drug.

• Potential for buccal delivery in paediatric and geriatric patients.
Acknowledgements

• Commonwealth Scholarships Commission

• Dr. Joshua Boateng
• Prof. John Mitchell
• Dr. Ian Slipper
• Dr. Concetta Giovino
• Dr. Farnoosh Kianfar
Thank you

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