Influence of chitosan on a polysaccharide blend *in situ* gelling powder for wound dressing

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Wound dressing

Cutaneus ulcers
- Lower limb ulcers
- Diabetic foot
- Bedsores

Conventional dressings
- Local irritation, contact sensitization and immune reactions
- Frequent dressing changes
- Dehydration of the wound bed
- Traumatic removal

Ideal wound dressing
- Absorption of exudate
- Transpiration
- Adhesiveness to the wound site
- Easy application and atraumatic removal
- Drug/adiuvant release
- Inexpensive

The global advanced wound dressing market was valued at USD 6.32 billion in 2018 and it is predicted to reach an CAGR (Compound Annual Growth Rate) of 4.3% over the forecast period.
In situ gelling powders

Micro particle carriers in form of dry powders

- Easy application
- Absorption of exudate
- Conformability to the surface of the wound
- Atraumatic removal
- Release of active pharmaceutical ingredients (API)

Production by mini spray drying

Economic Biopolymer

Alginate with high content of M induce the production of cytokines by human monocytes, a very useful process in the healing of chronic wounds.

Amidated pectin with a low degree of methylation is able to increase in situ gel forming rate.

Chitosan, antimicrobial agent, activates macrophages, stimulates cell proliferation and tissue organization. Chitosan low molecular weight enhance in situ gelification.


Alginate/pectin/chitosan powders

- Process yield depends on chitosan concentration
- Mean diameter is related to the concentration of chitosan

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Polymers concentration (w/V)</th>
<th>Polymers ratio</th>
<th>Yield (%)</th>
<th>Mean diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC_111</td>
<td>0.15</td>
<td>1:1:1</td>
<td>62.12</td>
<td>7.23</td>
</tr>
<tr>
<td>APC_113</td>
<td>0.15</td>
<td>1:1:3</td>
<td>73.14</td>
<td>2.43</td>
</tr>
<tr>
<td>APC_117</td>
<td>0.15</td>
<td>1:1:7</td>
<td>73.57</td>
<td>2.74</td>
</tr>
</tbody>
</table>

- Increasing of chitosan leads to an higher surface roughness
Alginate/pectin/chitosan powders

- **Fluid uptake ability:** maximum swelling in about 5 minutes

**Conditions:** Franz cell filled with SWF (simulated wound fluid), 37°C
• **Cytotoxicity activity**
  - Cells used: human keratinocyte (HaCAT)
  - Concentration of powders tested = 0.01-10 µg/mL

MTT test did not show any significant cytotoxic activity
Biological test

- **Proinflammatory activity**
  - Cells used: Human Epidermal Keratinocyte, adult (HEKa)
  - Concentration of powders tested = 0.1;0.5;1 µg/mL

![Graphs showing cytokine release](image)

APC induced a higher release of Interleukin-8
In situ gelling powders loaded doxycycline

- Wide antibacterial spectrum against Gram-positive and Gram-negative bacteria *
- Inhibition of host matrix metalloproteinases hyper expressed in chronic wounds **


Alginate/pectin/chitosan powders loaded doxycycline

- **Encapsulation efficiency (e.e.)** depending on the relative amount of chitosan into the feed

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Polymers concentration (w/V)</th>
<th>Polymers ratio</th>
<th>Doxycycline concentration % (w/w)</th>
<th>Yield (%)</th>
<th>Mean diameter (nm)</th>
<th>Drug content (%)</th>
<th>E.E. (%)</th>
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</thead>
<tbody>
<tr>
<td>d) APCD_111_2D</td>
<td>0.15</td>
<td>1:1:1</td>
<td>2</td>
<td>62.1</td>
<td>9.64</td>
<td>1.32</td>
<td>67.39</td>
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<tr>
<td>e) APCD_113_2D</td>
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<td>1:1:3</td>
<td>2</td>
<td>70.82</td>
<td>3.09</td>
<td>1.42</td>
<td>71.73</td>
</tr>
<tr>
<td>f) APCD_117_2D</td>
<td>0.15</td>
<td>1:1:7</td>
<td>2</td>
<td>73.88</td>
<td>2.43</td>
<td>1.51</td>
<td>77.24</td>
</tr>
</tbody>
</table>

• Images of the encapsulated powders.
Alginate/pectin/chitosan powders loaded doxycycline

- **Fluid uptake ability**: APCD_117_2%D showed a lower swelling than blank formulation
Doxycycline release

- **In vitro doxycycline release**
  - Similar trend for APCD 117_2%D and APCD 113_2%D
  - Higher amount of doxycycline released for APCD 111_2%D
**Antimicrobial test**

- Disc diffusion assay on *Staphylococcus aureus* (ATCC 6538)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of doxy (µg)</th>
<th>Area (mm²)</th>
<th>Amount of doxy N (µg)</th>
<th>Area N (mm²)</th>
<th>Δ area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxy</td>
<td>1.55</td>
<td>759.36</td>
<td>1.55</td>
<td>759.36</td>
<td></td>
</tr>
<tr>
<td>APCD_111_2D</td>
<td>1.59</td>
<td>780.83</td>
<td>1.55</td>
<td>761.18</td>
<td>0.24%</td>
</tr>
<tr>
<td>APCD_113_2D</td>
<td>1.58</td>
<td>878.88</td>
<td>1.55</td>
<td>862.19</td>
<td>13.54%</td>
</tr>
<tr>
<td>APCD_117_2D</td>
<td>1.55</td>
<td>934.29</td>
<td>1.55</td>
<td>934.29</td>
<td>23.04%</td>
</tr>
</tbody>
</table>
**Biological test**

- **SDS-PAGE gelatin zymography**: doxycycline released from hydrogel inhibited MMP-2 even at 0.5 μg and its effect was stable up to 72 h

![Graph showing MMP-2 inhibition by Doxy and APCD_111_2D](image)

- Graph showing densitometry process/control (%) for MMP-2 with Doxy and APCD_111_2D treatments.
Conclusions

- Chitosan affected the particles properties leading to better characteristics.
- Formulations did not show cytotoxic activity inducing IL-8 release at the application site.
- All formulations showed a prolonged release of doxycycline enabling higher efficacy against bacteria and inhibiting MMP-2 activity.
Thank you for the attention

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