STEP ITN Lectures

Marie Curie Initial Training Network

Shaping and Transformation in the Engineering of Polysaccharides (STEP)

Steve Harding, NCMH
University of Nottingham
STEP ITN Lectures

September 2009: Hydrodynamic characterisation of macromolecules

http://www.stepitn.eu/?page_id=1113

February 2010: Sizes, shapes & interactions of molecules in solution
• Albert Einstein and the Viscosity of Macromolecules
• Light Scattering and SEC-MALLs
• Dynamic Light Scattering
• Analytical Ultracentrifugation I
• Analytical Ultracentrifugation II: Interactions

http://www.stepitn.eu/?page_id=1137

June-July 2010: From sticky mucus to probing our past: Aspects and problems of the Biotechnological use of Macromolecules

http://www.chemie.uni-jena.de/institute/oc/heinze/Lecture_harding.html
From Sticky Mucus to Probing our Past: Aspects and problems of the Biotechnological use of Macromolecules

<table>
<thead>
<tr>
<th>Datum/Zeit</th>
<th>Veranstaltungsort</th>
<th>Thema</th>
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<tbody>
<tr>
<td>Mi, 30.06.2010</td>
<td>SR 309 Carl-Zeiss-Str. 3</td>
<td><strong>Macromolecules as BioPharma mucoadhesives</strong></td>
</tr>
<tr>
<td>12.15-13.45</td>
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<td>Do, 01.07.2010</td>
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<td>HS Haus 1 August-Bebel-Str. 2</td>
<td><strong>Stability in response to Bioprocessing I.</strong></td>
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<td><strong>Thermal Processing, D, z and F values</strong></td>
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<tr>
<td>Fr, 02.07.2010</td>
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<td><strong>Stability in response to Bioprocessing II: Irradiation</strong></td>
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<td><strong>and freezing</strong></td>
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<tr>
<td>Fr, 02.07.2010</td>
<td>SR 307 Carl-Zeiss-Str. 3</td>
<td><strong>The use of non-recombining parts of the</strong></td>
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<td><strong>Y-chromosomal DNA and mitochondrial DNA as a probe into</strong></td>
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<td></td>
<td></td>
<td><strong>our past</strong></td>
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From Sticky Mucus to Probing our Past: Aspects and problems of the Biotechnological use of Macromolecules

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Macromolecules as BioPharma mucoadhesives

Steve Harding
STICKY MUCUS IS IMPORTANT!

Consider a typical animal, zum beispiel - a Slug:
“If its love that makes the world go round”
“If its love that makes the world go round”

“then its mucus and slime which keeps it in perpetual motion”
Mucus

(i) adherent mucus gel in human GI tract

(ii) mucin glycoprotein

Pig colonic mucin: Jumel et al, 1997
Polysaccharides - from jam, jellies,

Mucus Glycoproteins - "mucins"
Mucus

(i) adherent mucus gel in human GI tract

(ii) mucin glycoprotein

Pig colonic mucin: Jumel et al, 1997
Electron microscopy of bronchial mucins. Harding, Rowe and Creeth, 1983
Mucins have a very broad molecular weight distribution…

Jumel et al, 1997
# Mucin types

<table>
<thead>
<tr>
<th>Mucin</th>
<th>Main expression</th>
<th>Chromosome</th>
<th>Amino acids in Tandem repeat*</th>
<th>Does it gel?</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC1</td>
<td>Breast, Pancreas</td>
<td>1</td>
<td>20</td>
<td>NO</td>
</tr>
<tr>
<td>MUC2</td>
<td>Intestine, Tracheobronchus</td>
<td>11</td>
<td>23*</td>
<td>YES</td>
</tr>
<tr>
<td>MUC3</td>
<td>Intestine, gall</td>
<td>7</td>
<td>7</td>
<td>YES</td>
</tr>
<tr>
<td>MUC4</td>
<td>Colon, Tracheo,</td>
<td>3</td>
<td>16</td>
<td>YES</td>
</tr>
<tr>
<td>MUC5A/C</td>
<td>Cervix</td>
<td>11</td>
<td>8</td>
<td>YES</td>
</tr>
<tr>
<td>MUC5B</td>
<td>Stomach, Tracheo, Cervix, Eye</td>
<td>11</td>
<td>29</td>
<td>YES</td>
</tr>
<tr>
<td>MUC6</td>
<td>Tracheo, Salivary</td>
<td>11</td>
<td>169</td>
<td>YES</td>
</tr>
<tr>
<td>MUC7</td>
<td>Stomach, gallbladder</td>
<td>4</td>
<td>23</td>
<td>NO</td>
</tr>
<tr>
<td>MUC8</td>
<td>Salivary</td>
<td>12</td>
<td>41</td>
<td>YES</td>
</tr>
</tbody>
</table>

*MUC 2 tandem repeat: PTTPITTTTVPPTPTPTGTQT. MUC3: HSTPSFTSSITTETTS. MUC4: TSSASTGHATPLPVTID; MUC5A/C: TTSTTSAP

There are ~ 17 MUC genes now identified.
Mucin sugars

- Fucose
- GalNAc
- GlcNAc
- Galactose
- Sialic acid
Mucin sugars

Sticky bits
Mucin sugars

Sticky bits
Mucin sugars

Sticky bits
So, mucins are:

1. Large, hydrated, polydisperse, flexible coil
2. 80-90% glycosylated: key sites for interaction on sugars
3. Electrostatic sites: sialic acid (and also possible sulphonated groups)
4. Hydrophobic: fucose
Oral drug administration

- most popular with medical staff & patients
- majority of drug absorbed at small intestine (~100m²)
- clearance time though generally too short (4-12h)
Oral drug administration

Low appearance of drug due to

• too rapid a transit past the ideal absorption site

• rapid degradation in the g.i. tract once released

• low transmucosal permeability
Oral drug administration

Low appearance of drug due to
• too rapid a transit past the ideal absorption site

Macromolecular brakes: MUCOADHESIVES
Now: The mucoadhesive

- non-toxic & not expensive
- high drug loading capacity
Now: The mucoadhesive

- non-toxic & not expensive
- high drug loading capacity

POLYSACCHARIDES
**Ingredients:** Potassium Nitrate (5%), Stannous Fluoride (0.45%), Glycerin And/Or Sorbitol, Water, Hydrated Silica, PEG-40 Castor Oil, PEG-12, Sodium Bicarbonate, Sodium Lauryl Sulfate, Poloxamer 407, Sodium Citrate, Flavor, Titanium Dioxide, Sodium Hydroxide, **Cellulose Gum**, **Xanthan Gum**, Sodium Saccharin, Stannous Chloride, Citric Acid, Tetrasodium Pyrophosphate, FD&C Blue #1, D&C Yellow #10

**Ingredients:** Polymethyvinylether Maleic Acid Calcium-Zinc Salt, Petrolatum, Mineral Oil, **Cellulose Gum**, Silicon Dioxide, Flavor, Red 27 Aluminum Lake
Mucin sugars
### Mucoadhesive performance: Tensiometric analysis

**{CM Lehr, JA Boustra, EH Schacht, HE Junginger (1992) Int J Pharm.78, 43-48}**

<table>
<thead>
<tr>
<th>Neutral polysacces</th>
<th>$F$ (mN/cm²)</th>
<th>Chitosans</th>
<th>$F$ (mN/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-cellulose</td>
<td>~0 (2.8±2.8)</td>
<td>Wella low-visc.</td>
<td>3.9±1.2</td>
</tr>
<tr>
<td>HE-starch</td>
<td>~0 (0.6±0.8)</td>
<td>Wella high-visc</td>
<td>6.7±0.7</td>
</tr>
<tr>
<td>Scleroglucan</td>
<td>~0</td>
<td>Knaepzyk</td>
<td>5.7±1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Daichitosan-H</td>
<td>8.0±5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Daichitosan-VH</td>
<td>9.5±2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sea-Cure 240</td>
<td>4.1±2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sea-Cure 210+</td>
<td>9.5±2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sigma</td>
<td>6.6±3.0</td>
</tr>
<tr>
<td>Anionic polysacces</td>
<td></td>
<td>Cationic dextrans</td>
<td></td>
</tr>
<tr>
<td>Pectin</td>
<td>~0</td>
<td>DEAE-dextran</td>
<td>~0</td>
</tr>
<tr>
<td>Xanthan</td>
<td>~0</td>
<td>Amino-dextran</td>
<td>~0</td>
</tr>
<tr>
<td>CMC (low visc)</td>
<td>1.8±1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMC (medium)</td>
<td>0.3±0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMC (high visc)</td>
<td>1.3±1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Image Descriptions:**
- **Left:** Diagram of a substrate coated surface.
- **Center:** Diagram illustrating mucoadhesive force ($F$).
- **Right:** Diagram showing the force ($F$) applied to a coated surface.
Molecular assay methods

- Viscometry/rheology
- Surface plasmon resonance
- Dynamic light scattering
- Turbidity/light scattering
- SEC-MALLS/FFF-MALLS
- Analytical ultracentrifuge
- Electron microscopy
- Atomic Force Microscopy
Molecular assay methods

- Viscometry/ rheology
- Surface plasmon resonance
- Dynamic light scattering
- Turbidity/ light scattering
- SEC-MALLS/ FFF-MALLS
- **Analytical ultracentrifuge**
- Electron microscopy
- Atomic Force Microscopy
Dr. Conny Jumel
Modern set-up:

- Viscosity detector
- Concentration detector
- MALLs detector
- Columns
Molecular weight distribution for colonic mucin

Molecular assay methods

- Viscometry/ rheology
- Surface plasmon resonance
- Dynamic light scattering
- Turbidity/ light scattering
- SEC-MALLS/ FFF-MALLS
- Analytical ultracentrifuge
- Electron microscopy
- Atomic Force Microscopy
Optima XLA / XLI
Sedimentation Velocity in the Analytical Ultracentrifuge

Centrifugal force

Top view, sector of centrifuge cell

Air
Solvent
Solution

conc, c

Rate of movement of boundary \( \rightarrow \) sed. coeff

distance, r

\( S_{20,w}^0 = 10^{-13} \text{ sec} \)
Glue Protein

Direction of sedimentation

0.8mg/ml
40,000 rpm, Scan every 10 min

$s_{20,w} = 2.3$ Svedbergs
Mussels ➔ “Glue foot protein” mefp1
Mussel Glue Protein Hydrodynamics

\[ M = 110,000 \text{ g/mol} \]

- : non-repetitive globular region

\~\ : flexible segment \([\text{P}^*\text{P}^*\text{TYK}]\)

\_- : rigid segment \([\text{AKPSY}]\)

Deacon, Waite, Davis & Harding, Biochemistry, 1998
Glue Protein

Direction of sedimentation

0.8mg/ml
40,000 rpm, Scan every 10 min

\[ s_{20,w} = 2.3 \text{ Svedbergs} \]
Glue Protein + Mucin

Glue protein: 0.4mg/ml
Mucin: 0.1mg/ml (invisible)

2000 rpm, Scan every 10min

$s_{20,w} \sim 7,000$S
Candidate Polysaccharides:

- Guar
- Alginate
- Carboxy-methyl cellulose
- Xanthan
- DEAE-dextran
- Chitosans
Candidate Polysaccharides:

Guar
Alginate
Carboxy-methyl cellulose
Xanthan
DEAE-dextran
Chitosans
Simple criterion for an interaction:

\[
\text{RATIO:} \quad \frac{\text{Sedimentation value of complex}}{\text{Sedimentation value of mucin}}
\]
**DEAE dextran**

<table>
<thead>
<tr>
<th>mucin:DEAE-dextran ratio</th>
<th>Buffer+temp</th>
<th>$s_{\text{mucin}}$ (S) control</th>
<th>$s_{\text{mix}}$ (S) complex</th>
<th>$s_{\text{mix}}/s_{\text{mucin}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0: 1.9 (mg/ml)</td>
<td>pH6.8, I=0.1, 20 °C</td>
<td>17</td>
<td>19</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>“” “” 37 °C</td>
<td>17</td>
<td>20</td>
<td>1.2</td>
</tr>
<tr>
<td>1.8: 3.2</td>
<td>“” “” “”</td>
<td>18</td>
<td>25</td>
<td>1.4</td>
</tr>
<tr>
<td>0.2: 1.0</td>
<td>“” “” 20 °C</td>
<td>35</td>
<td>65</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>pH7.0 tris “”</td>
<td>42</td>
<td>55</td>
<td>1.3</td>
</tr>
</tbody>
</table>

**DEAE-Dextran**

n~500
Crabshell: \(\Leftrightarrow \text{chitin} \Leftrightarrow \text{chitosan}\)
Sedimentation velocity assay: mucin+chitosans

UV absorption optics (Beckman XL-A ultracentrifuge)
chitosan controls: sed. coeff. \( s \approx 1.5 \) Svedbergs (S)
mucin: chitosan ratio 0.2mg/ml : 1.0mg/ml
Sea-Cure +210 (Pro-Nova, Drammen): degree of acetylation \( F_A \approx 0.11 \)
KN50 (NTH-Trondheim) “ “ “ “ \( F_A \approx 0.42 \)

<table>
<thead>
<tr>
<th>chitosan</th>
<th>Buffer+temp</th>
<th>( s_{\text{mucin}} ) (S) control</th>
<th>( s_{\text{mix}} ) (S) complex</th>
<th>( s_{\text{mix}}/s_{\text{mucin}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>sea-cure +210</td>
<td>pH4.5, I=0.1, 20^\circ C</td>
<td>53</td>
<td>780</td>
<td>15</td>
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<td></td>
<td>“ “ 37^\circ C</td>
<td>53</td>
<td>1990</td>
<td>38</td>
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<tr>
<td>KN50 Trondheim</td>
<td>pH4.5, I=0.1, 20^\circ C</td>
<td>53</td>
<td>1630</td>
<td>31</td>
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<tr>
<td></td>
<td>“ “ 37^\circ C</td>
<td>53</td>
<td>2340</td>
<td>44</td>
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Images of mucin/chitosan complexes using Electron Microscopy

Mucin-chitosan

Mucin- Gold labelled chitosan

Fiebrig et al, 1996
Images of mucin/chitosan complexes using Atomic Force Microscopy

Deacon, McGurk, Roberts, Williams, Tendler, Davies, Davis & Harding (2000), Biochem. J. 348, 557
Atomic force microscopy: mucin

a&c: topography mode
b&d: phase mode

Deacon, McGurk, Roberts, Williams, Tendler, Davies, Davis & Harding (2000), Biochem. J. 348, 557
Atomic force microscopy: chitosan

a&c: topography mode

b&d: phase mode

Deacon, McGurk, Roberts, Williams, Tendler, Davies, Davis & Harding (2000), Biochem. J. 348, 557
Sedimentation velocity assay: mucin+chitosan (sea cure 210+) Effect of pH

<table>
<thead>
<tr>
<th>pH</th>
<th>Temp</th>
<th>$s_{mucin}$ (S) control</th>
<th>$s_{mix}$ (S) complex</th>
<th>$s_{mix}/s_{mucin}$</th>
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<tbody>
<tr>
<td>2.0</td>
<td>20°C</td>
<td>45</td>
<td>980</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>37°C</td>
<td>132</td>
<td>1626</td>
<td>12</td>
</tr>
<tr>
<td>4.5</td>
<td>20°C</td>
<td>53</td>
<td>780</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>37°C</td>
<td>53</td>
<td>1990</td>
<td>38</td>
</tr>
<tr>
<td>6.5</td>
<td>20°C</td>
<td>32</td>
<td>1524</td>
<td>48</td>
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<tr>
<td></td>
<td>37°C</td>
<td>46</td>
<td>1580</td>
<td>34</td>
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Sedimentation Fingerprinting: assay for mucoadhesive interaction where mucin concentration is too small to be detected

Schlieren Optical system:

Chitosan-mucin mixture:

Chitosan control:

Loss of concentration of chitosan through complex formation as index for interaction
Chitosan-mucin interactions specific to different regions of the stomach

Deacon, Davis, White, Nordman, Carlstedt, Errington, Rowe & Harding, 1999

Chitosan: $F_A = 0.11$, Initial concentrations: chitosan 4 mg/ml, mucin <1 mg/ml, I=0.1M
Oral formulations need nanoparticles/microparticles

Prepared with tripolyphosphate at pH 5.3 with insulin loading concentration of 4.28 μg/ml.

Oral drug administration?
Oral drug administration?

What about the nose?
Mucoadhesion in the nose
Chitosan and nasal delivery

• Decreased Clearance Rate
  chitosan is a mucoadhesive material

• Effect on inter-cellular transport
  transient opening of “tight junctions” has been shown in cell cultures
Clearance of Chitosan Formulations from the Nasal Cavity of Man (n=4)

Illum, L. et al, 2002
Nasal administration of Insulin to Sheep with chitosan

Illum, L. et al, 2002
Mucoadhesion: work in progress

- Efficiency of chitosan based encapsulation systems
- Stability of chitosan
STABILITY OF CHITOSAN FORMULATIONS – viscosity is good here!

*Chitosan: CL210 (F_A = 0.18), Effect of temperature on storage*
Chitosan: CL210 ($F_A = 0.18$), Effect of ionic strength on storage

Danke! - Thank you for your attention!
Some references


All refs (apart from #5) can be accessed from http://www.nottingham.ac.uk/ncmh/harding_publish.html