LIVE WEBINAR: A better understanding of gelatin cross-linking: causes, control strategy and regulatory approaches.

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ABOUT PROCAPS

- Latin America's largest softgel producer with facilities in Brazil, Colombia and Venezuela, and exports reaching more than 38 countries worldwide.
- Deep knowledge in gelatin formulation and an impressive scientific expertise in softgel technologies.
- Specialized in innovative softgel associated technologies such as Unigel™, Versagels™, G-tabs™ & Gummygels™.
- We cater to multinational pharmaceutical clients, including Pfizer, GlaxoSmithKline, Sanofi-Aventis, Boehringer Ingelheim, and Bayer among others.
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1. Introduction
2. Collagen and Gelatin
3. Cross-Linking Chemistry
4. Factors Affecting Cross-Linking
5. Quality and Regulatory Considerations
1. Introduction
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Introduction

Gelatin owns five unique properties:
1) It is not toxic.
2) Soluble at corporal temperature.
3) Forms resistant and flexible pellicles.
4) Flows in solution at about 50°C.
5) Reversible transition from sol to gel.

During storage, gelatin included in dosage forms can suffer a series of chemical reactions named cross-linking.

Cross-linking in gelatin can affect *in vitro* the dissolution rate of the capsule outer and therefore, the release and dissolution of the drug substance contained in the fill content.

Cross-Linking frequently differs in soft shell capsules from hard shell capsules because of the larger mass of gelatin in the soft shell dosage form.

Does cross-linking affect significatively in *vivo* performance of the dosage form?
1. Introduction

2. Collagen and Gelatin

3. Cross-Linking Chemistry

4. Factors Affecting Cross-Linking

5. Quality and Regulatory Considerations
Collagen and Gelatin

Collagen is the main structural protein of the various connective tissues in animals.
The irreversibility differentiates gelatin from soluble collagen or hydrolysates.

Collagen is insoluble due to inter and intra molecular cross-linking.

Reversible process. It depends on temperature and polymer concentration.

The irreversibility differentiates gelatin from soluble collagen or hydrolysates.

Source: http://www.nitta-gelatin.co.jp/
Collagen and Gelatin

The two types of gelatin are characterized by their mode of manufacturing.

Type A Gelatin (Acid Processing)
- Raw Material Receiving (Pork Skin)
  - Chopping
  - Acid Treatment
  - Extraction
  - Filtration
  - Concentration
  - Deionization
  - Drying
  - Milling & Sifting
  - Quality Testing
  - Blending & Packaging
  - Final Inspection
  - Shipping

Type B Gelatin (Alkaline Processing)
- Raw Material Receiving (Beef Bones)
  - Milling
  - Milling, Sifting & Blending
  - Lime Treatment
  - Drying
  - Extraction
  - Chilling
  - Packaging
  - Filtration
  - Sterilization
  - Final Inspection
  - Deionization
  - Concentration
  - Shipping

Source: http://www.nitta-gelatin.co.jp/

pH: 3.8-6.0
Isoelectric point: 6-8

pH: 5.0-7.4
Isoelectric point: 4.7-5.3
Collagen and Gelatin

Gelatin composition is closer to collagen but some variations occur depending on the manufacturing process (acid or basic hydrolysis).

Table 1. Amino acid composition of bovine and porcine skin gelatin

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>BSG (residues per 1000 total amino acid residues)</th>
<th>PSG (residues per 1000 total amino acid residues)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpolar hydrophobic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>33</td>
<td>80</td>
</tr>
<tr>
<td>Valine</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Leucine</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Methionine</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Proline</td>
<td>63</td>
<td>151</td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>335</td>
</tr>
<tr>
<td>Polar uncharged</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>108</td>
<td>239</td>
</tr>
<tr>
<td>Threonine</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td>307</td>
</tr>
<tr>
<td>Polar acidic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>17</td>
<td>41</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>34</td>
<td>83</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>124</td>
</tr>
<tr>
<td>Polar basic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>Arginine</td>
<td>47</td>
<td>111</td>
</tr>
<tr>
<td>Histidine</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>138</td>
</tr>
</tbody>
</table>

Table 2. Gel strength of BSG and PSG at different pHs

<table>
<thead>
<tr>
<th>pH</th>
<th>BSG</th>
<th>PSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>193.49 ± 2.098</td>
<td>330.57 ± 0.811</td>
</tr>
<tr>
<td>4</td>
<td>234.00 ± 0.469</td>
<td>372.05 ± 0.45</td>
</tr>
<tr>
<td>5</td>
<td>331.04 ± 1.062</td>
<td>366.47 ± 0.671</td>
</tr>
<tr>
<td>6</td>
<td>264.69 ± 5.874</td>
<td>350.32 ± 1.055</td>
</tr>
<tr>
<td>7</td>
<td>267.03 ± 8.016</td>
<td>389.64 ± 0.29</td>
</tr>
<tr>
<td>8</td>
<td>270.35 ± 8.024</td>
<td>415.30 ± 1.211</td>
</tr>
<tr>
<td>9</td>
<td>247.02 ± 1.808</td>
<td>348.57 ± 2.366</td>
</tr>
</tbody>
</table>

CONTENT

1. Introduction
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3. Cross-Linking Chemistry
4. Factors Affecting Cross-Linking
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Cross-Linking Chemistry

In the structure shown below (top left), R represents a side-chain specific to each amino acid.

Figure 1.

Figure 2.

Reactivity of “R” chain, will determine reactivity of gelatin to cross-linking reactions.

Figure 3.

Glycine

Lysine
Basic amino acids, specially Lysine and Arginine, are more reactive in reactions that involve cross-linking.

Source: http://content.learntoday.info/Thuze/Biology/content/ch15.html
Cross-Linking Chemistry

Self Cross-Linking

No external aldehydes involved

Cross-linked gelatin
Cross-Linking Chemistry

Chemically Induced Cross-Linking

Cross-Linking of Gelatin Capsules and Its Relevance to Their in Vitro-in Vivo Performance

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Cross-Linking Chemistry

Chemically Induced Cross-Linking
Cross-Linking Chemistry

Chemically Induced Cross-Linking

Cross-Linking of Gelatin Capsules and Its Relevance to Their in Vitro–in Vivo Performance

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Cross-Linking Chemistry

Depending on the involved reactions, cross-linking is evidenced on different part of the capsules:

- Shell
  - Self Cross-Linking
  - Chemically Induced Cross-Linking

- Gelatin shell becomes insoluble by exposure to T and RH
- Insoluble membrane between fill and shell (PELLICLE)

Both types of cross-linking can occur at the same time!
Cross-Linking Chemistry

PELLICLES

STICKY MASS

Source: USP Workshop, Maryland 2014.
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Factors Affecting Cross-Linking.

Cross-Linking

1. Gelatin
2. Drug Substance
3. Excipients: fill and shell
4. Packaging
5. Storage Condition

Type I factors: These factors can be modified by formulators.

Type II factors: These factors normally need to remain as they are.
Factors Affecting Cross-Linking:

Gelatin

- Bloom Adjustment
- Hydrolysis
- Collagen Age
- Tissue

Even gelatins with similar Bloom, could have differences in some other properties
Factors Affecting Cross-Linking: Gelatin

Polling Question #1

Which of the following tests, would you use to control gelatin variability due to either collagen age and/or Bloom adjustments?

a) Bloom

b) Viscosity

c) Bloom and Viscosity

d) Others
Factors Affecting Cross-Linking: Gelatin

Controlling gelatin grade at bloom level, could not be sufficient.

The molecular weight distribution (MWD) of one type A (■) and one type B (□) gelatin that exhibit comparable Bloom values (Haug & Draget, 2009a).
Factors Affecting Cross-Linking: Gelatin

Three different batches of the same gelatin were selected:

- Mw is the weight average-molecular weight. There is an equal weight of molecules on either side of Mw in the distribution.
- S3 showed lower percentage of high molecular weight chains compared to either S1 or S2.
Factors Affecting Cross-Linking: Gelatin: Case Study

- A batch of soft capsules was manufactured with one of the materials (S1, S2 and S3).
- 5 mg Yellow No. 6 in PEG-400 was used as a fill content.
- Gelatin Formulation consisted in 42% of each gelatin, 20% of glycerin and 38% of water.
- Capsules of each product were packaged in HDPE bottles.
- Packaged products were exposed for about 30 days at accelerated (40°C at 75% RH) conditions.
- Dissolution profiles for Yellow No. 6 were tested, at initial and after the about mentioned period at accelerated conditions.
- Dissolution conditions were: Apparatus 2, at 50 rpm, and using 900 mL of water as the dissolution media.
Factors Affecting Cross-Linking: Gelatin

Results for the three batches at time zero:

S1, Mw = 54,000 KDa
S2, Mw = 84,000 KDa
S3, Mw = 46,000 KDa

Earlier sampling points are more discriminating for dissolution rate.

At Initial, the lowest Mw for the used gelatin, the fastest the dissolution rate.
Factors Affecting Cross-Linking: Gelatin

Results for the three batches after 30 days at 40°C/75% RH:

- S1, $M_w = 54,000$ KDa
- S2, $M_w = 84,000$ KDa
- S3, $M_w = 46,000$ KDa
Factors Affecting Cross-Linking: Gelatin

Polling Question # 2

Which gelatin would you select?

a) S1
b) S2
c) S3
Factors Affecting Cross-Linking: Gelatin

Results for the three batches after 30 days at 40°C/75% RH:

- **S1**, Mw = 54,000 KDa
- **S2**, Mw = 84,000 KDa
- **S3**, Mw = 46,000 KDa

There should be something additional to MWD, which affects the reactivity of gelatin to cross-linking during storage.
Factors Affecting Cross-Linking:

Gelatin

Summary of gelatin effects:

- Manufacturing of gelatin lead into a high variability that cannot be detected by most of the traditional tests.

- Bloom is not enough to characterize gelatin in relation with source and MWD.

- MWD appears as an important parameter to be discussed with your gelatin supplier.

- Lower MWD leads into gelatin formulations that “open” faster, but it does not mean they would not be cross-linked during storage.

- Reactivity of gelatin to cross-linking should be evaluated. That reactivity should depend on gelatin composition.
Factors Affecting Cross-Linking: Drug Substance

Chemically induced cross-linking can be affected (promoted, retarded or unaffected), by functional group(s) of the drug substance.

Functional groups that promote cross-linking:
- Formaldehyde and carbonyl groups
- Glyceraldehyde
- Peroxides
- Benzensulfonic acid
- Guanidine HCl
- Dyes: Red No. 3, 40, and Blue No.1. Yellow No. 10.
- Trivalent ions (Al$^{3+}$)

Functional groups that retard cross-linking:
- Basic amino acids (Lysine, glycine)
- Hydroxylamine HCl
- P-aminobenzoic acid
- Semicarbazide
- Piperazine
- Pyridine

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Factors Affecting Cross-Linking: Drug Substance

Primary and secondary amino, work as scavengers for molecules that promotes cross-linking

Pre-formulation stage should include effect of drug substance on chemically induced cross-linking

The Influence of Drugs on Gelatin Cross-Linking
Deepthi Gholap; Saranjit Singh
Pharmaceutical Technology, Apr 2004, 28, 4; ABI/INFORM Global pg. 94
Factors Affecting Cross-Linking: Excipients

1. Auto-oxidation of Polyethylene Glycols and Polysorbates:

   ![Furfural Chemical Structure]

2. Essential oils like Peppermint oil:

   ![Carvone Chemical Structure]
   ![Pulegone Chemical Structure]
Factors Affecting Cross-Linking: Excipients

3. Hydrolysis of Hexamethylenetetramine:

4. Auto-oxidation of Polyethylene Glycols and Polysorbates:
Factors Affecting Cross-Linking: Excipients

Level of aldehydes in polyethylene glycols and similar materials, should be tightly controlled.
Factors Affecting Cross-Linking: Packaging and storage conditions

Cross-Linking is strongly accelerated by temperature but specially by high humidity.

Water permeation rate for packaging material should be carefully evaluated and used for packaging selection.

For example, better results can be obtained using Aclar rather than PVDC or PVC blisters.

Excessive exposure to light should be avoided.

Cross-linking is not uniform within a batch and even sometimes a single capsule evidences more in some portions: HIGH VARIABILITY OF RESULTS.
Factors Affecting Cross-Linking:
How to control cross-linking in my product?

Strategy needs to evaluate all factors and modify the ones that can be modified.
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# Quality and Regulatory Considerations:

<table>
<thead>
<tr>
<th>USP &lt;711&gt;</th>
<th>Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>- In the early 1990's a second dissolution method was included in USP General Chapter &lt;711&gt;. It includes enzymes in order to dissolve the Cross-Linked shell.</td>
<td>- Pepsin is only active until pH 4.0, not up to pH 6.8.</td>
</tr>
<tr>
<td>- Enzymes are selected according to pH of the Dissolution media.</td>
<td>- Pepsin activity method and units.</td>
</tr>
<tr>
<td>- Pepsin is selected were water or a media with a pH is lower than 6.8. Pancreatin is selected when the pH media is greater than 6.8.</td>
<td>- Methods were water is the media.</td>
</tr>
<tr>
<td>- Limits were defined based on a collaborative study including PF 24 (5).</td>
<td>- What to do with pHs lower than 6.8 and higher than 4.0.</td>
</tr>
<tr>
<td></td>
<td>- What to do if surfactant denatures enzymes.</td>
</tr>
<tr>
<td></td>
<td>- Using enzymes and regulatory considerations.</td>
</tr>
</tbody>
</table>
Quality and Regulatory Considerations:

<table>
<thead>
<tr>
<th>Changes in USP &lt;711&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>New USP General Chapter &lt;1094&gt;: “Dissolution Testing and Related Quality Attributes” PF 39 (5) and official in 1st supplement, August 2014. Updates also in USP &lt;1092&gt; and &lt;711&gt;.</td>
</tr>
<tr>
<td>Pepsin will be used just when pH is lower than pH 4.0 and limit (NMT 750,000 units), will be revised based on new procedure for activity test.</td>
</tr>
<tr>
<td>Bromelain (NMT 30 GDU/L) and papain (NMT 550,000 U/L), will be selected for pHs between 4.0 and 6.8.</td>
</tr>
<tr>
<td>Pancreatin will remain as the enzyme for media with pH higher than 6.8 but limit will be increased until 2000 protease until per liter.</td>
</tr>
<tr>
<td>If surfactants need to be used, options for pre-treatment should be performed in NMT 15 minutes included in the total time of the test.</td>
</tr>
<tr>
<td>Using enzymes should be justified to Agencies.</td>
</tr>
</tbody>
</table>
Q&A Session
Thank you and don’t forget to...

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