Cross-Linking of Gelatin Capsule Shells

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Agenda

- Gelatin and Cross-Linking
- USP Procedure pre-40(6)
- 40(6) Revisions to USP <711> and <2040>
- Failures
- Prevention of Cross-Linking

What is Gelatin?

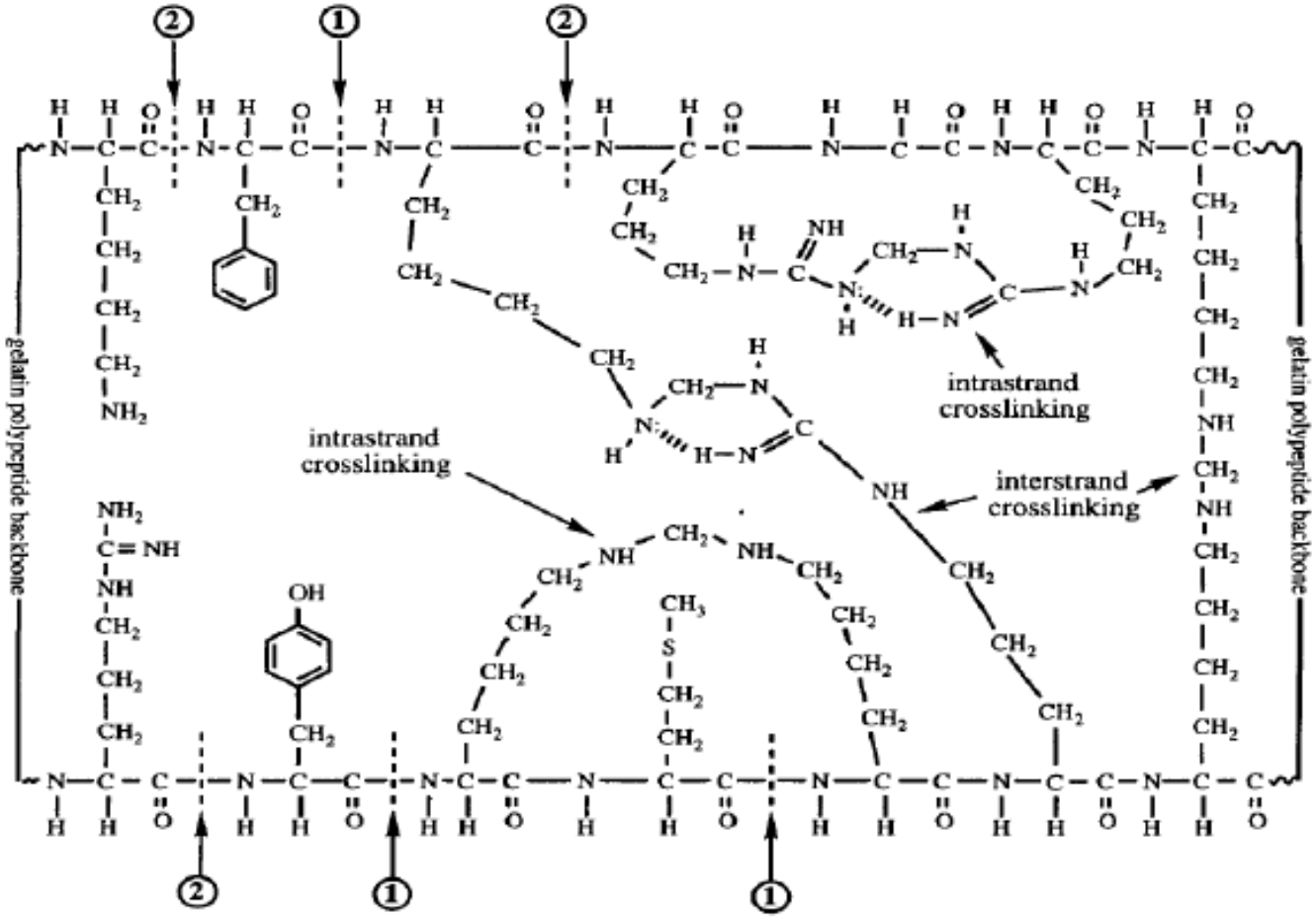
- Hydrolyzed form of collagen
- Collagen is obtained from various animal by-products
- Used as a gelling agents in a variety of applications:
  - Food
  - Cosmetics
  - Photography
  - Pharmaceuticals

What is Cross-Linking?

Cross-linking is the “formation of strong chemical linkages beyond simple hydrogen and ionic bonding between gelatin chains.”

- Reaction is generally irreversible
- Renders gelatin insoluble
- Reaction catalyzed by a number of chemical and environmental factors

*From USP Stimuli to the Revision Process, Use of Enzymes in the Dissolution Testing of Gelatin Capsules and Gelatin-Coated Tablets – Revisions. USP-PF 40(6)



① Site of possible peptide bond scission by pepsin
②Site of possible peptide bond scission by pancretin

J. Pharm. Sci. 1994, 83 (*7*): *915-921*

Gelatin Structure

- Protein with some potentially reactive side chains
- Often will form helices, etc.
- Can form covalent and hydrogen bonds between itself and another chain
- Cross-linking can occur in collagen as condensation products between aldehydes and amines, or between two aldehydes
- Gelatin more prone to cross-linking than collagen

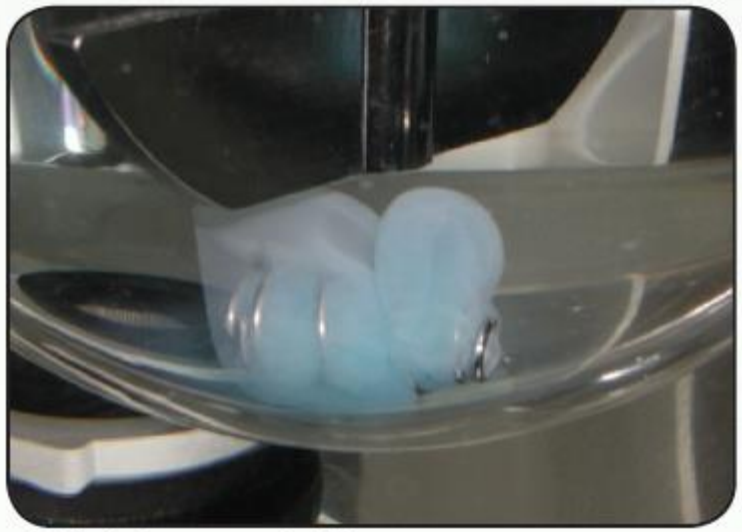
What can cause Cross-Linking in Gelatin?

- Aldehydes and Ketones
- APIs with carbonyl groups or potential aldehyde formation
- Oxidizing agents
- Metal Ions
- Sugars
- Heat
- High and Low Humidity
- Light
- Looking at it funny (just kidding – but it's very reactive)

Cross-Linking Impact

Capsule shell opening is delayed or stopped by pellicle formation on the internal or external gelatin surface

- Visually confirmed with seeing thin membranes or gelatinous masses
- Cross-linking amount can be determined by other methods – NMR, UV/Fluorescence Spectroscopy, MRI, Near IR Spectroscopy



Pellicle Formation Problem

Cross-Linking Impact

Lower and/or incomplete dissolution in vitro

In severe situations, can lead to problems in vivo as well

Cross-linking most commonly seen in stability testing

Pictures used courtesy of Vivian Gray, 2014

Current USP Approach to Cross-linking

USP <711>

"For hard or soft gelatin capsules and gelatin-coated tablets that do not conform to the Dissolution specification, repeat the test as follows."

- Media < pH 6.8 – repeat test with addition of purified pepsin (<750,000 units/L)
- Media ≥ pH 6.8 – repeat test with addition of pancreatin (<1750 USP units of protease activity/L)

Pepsin

- Digestive enzyme in stomach which converts proteins to peptides
- For cross-linking, this is used to clean gelatin proteins in places other than cross-linking site and allow rupture
- Typically obtained from glandular layer of hog stomach
- Peak activity at pH 2, good activity to ~pH 4.5

Pancreatin

- Mixture of digestive enzymes created by exocrine cells of pancreas
- Typically porcine/bovine origin
- Has good protease activity
- Peak Activity at pH 6.8, good between 6-8

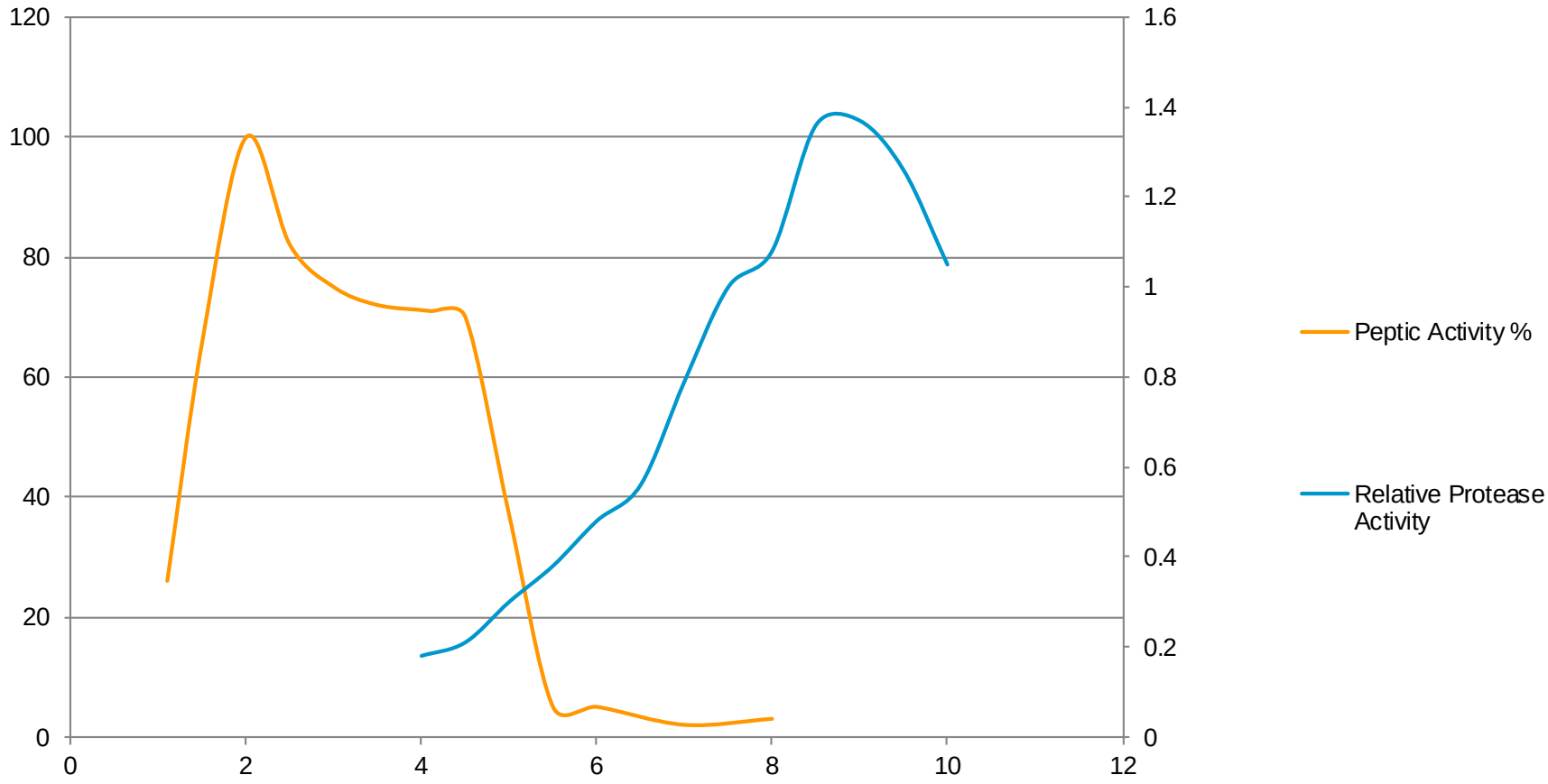
Success with USP <711>

- Pepsin activity generally felt sufficient to deal with mild to moderate cross-linking
- Pancreatin able to handle mild cross-linking

Limitations with USP <711>

- No good enzymes for pH 4-6.8
- Surfactant/Enzyme incompatibility
  - SLS and other surfactants can denature enzymes
  - Other enzymes can enhance activity
- Pancreatin levels too low?

Enzyme Activity in mid-range



Proposed Changes to <711>

Key Changes

- New Enzymes!
  - Papain
  - Bromelain
- Pre-treatment Option
- Increase in Pancreatin Levels
- Clarifications

When Enzymes are to be Used

<711> changed to indicate the enzymes are to be used only when:

- Product contains gelatin
- Product does not meet dissolution specification
- Evidence of cross-linking is observed

Prior to this, no evidence of cross-linking was required

Cross-Linking Evidence

Defined in USP <1094> Liquid-Filled Capsules – Dissolution Testing and Related Quality Attributes PF 38 (1)

- Observations (Gelatinous mass, pellicles, swelling w/o rupture)
- Instrumental Techniques (C13-NMR, FTIR, MRI, etc.)
- Switching capsule shells with fresh

Failure with Cross-Linking

If cross-linking is seen, and failure occurs you DO NOT need to continue testing until the last stage.

Papain

- Derived from unripe green papaya
- Commonly used as meat tenderizer, dietary supplement, etc.
- Digests protein substrates more extensively than pancreatin
- Optimal pH between 6-7 for most substrates, pH 5 for gelatin
- Powder stable 2 years at 2-8C

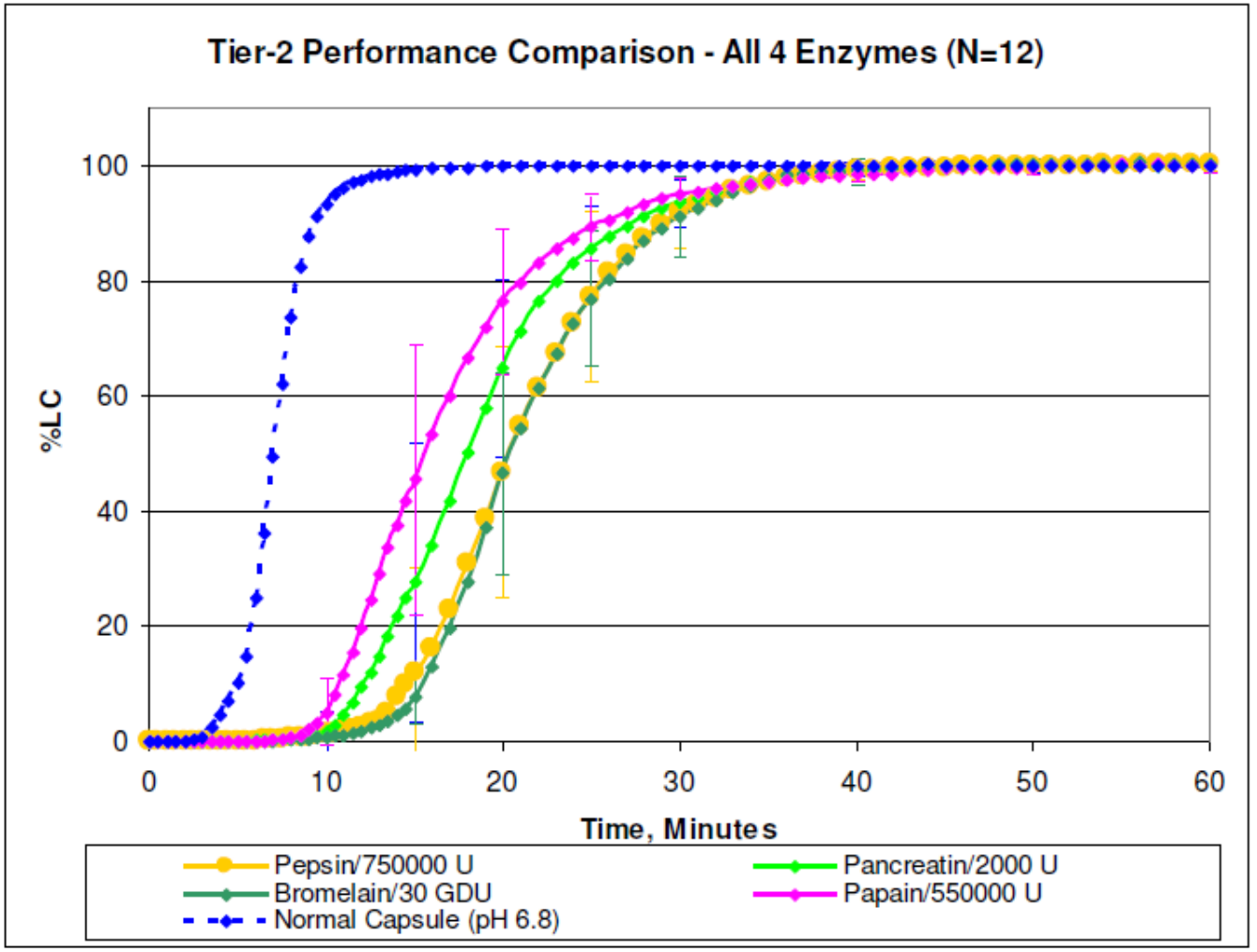
Bromelain

- From Pineapple Stems
- Used commonly as meat tenderizer, anti-inflammatory agent, leather tanning, etc.
- Optimal pH 5-7, stable from pH 3.0-6.5
- Powder stable 1.5-3 years at 2-8C

Papain and Bromelain in Dissolution

- Both suitable for use between pH 4-6.8
- Papain used in activity of NMT 550,000 Units/L
  - Activity determination explained in Papain monograph, under Assay
- Bromelain used in activity of NMT 30 gelatin-digesting units (GDU)/L of dissolution media
  - Activity determination determined in Reagent Specifications, Bromelain

New Enzyme Levels Equivalent to Pepsin



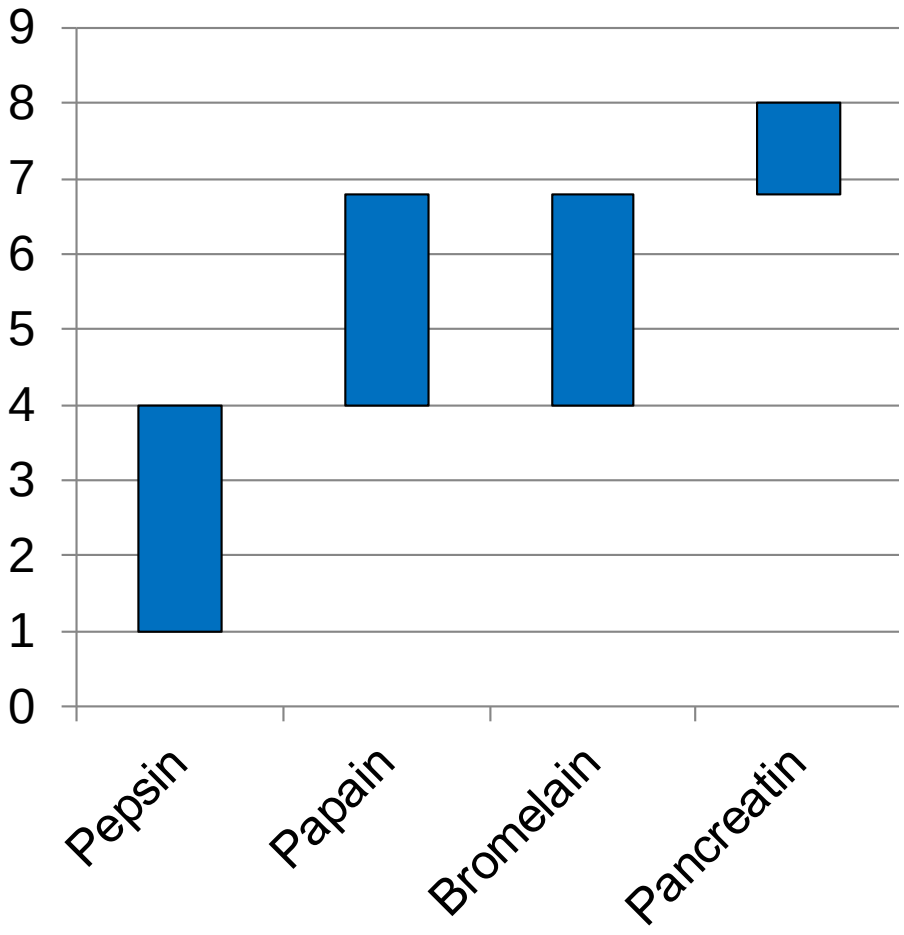
Used courtesy of Jian-Hwa Han, Abbvie

Increase in Pancreatin

Previous graph also shows reason for increase from 1750 to 2000 units/L

Pancreatin has been reported in the past to show a higher failure rate vs. pepsin in cross-linking

Enzyme Choice



- Pepsin now clarified for use with pH ≤ 4.0
- Pancreatin for use with pH ≥ 6.8
- For pH 4.0 – 6.8, you can use either Papain or Bromelain

Points of Consideration

- Enzyme interaction with media/formulation should be confirmed with samples through method development
- Enzyme(s) should be shown to be effective vs. cross-linked samples
  - Tier 1 vs. Tier 2 with same stressed samples (see USP <1094>)
  - Confirm performance of enzyme(s) to give proper results
  - Use enzyme with best activity
- Validation of a method should include enzyme use where appropriate

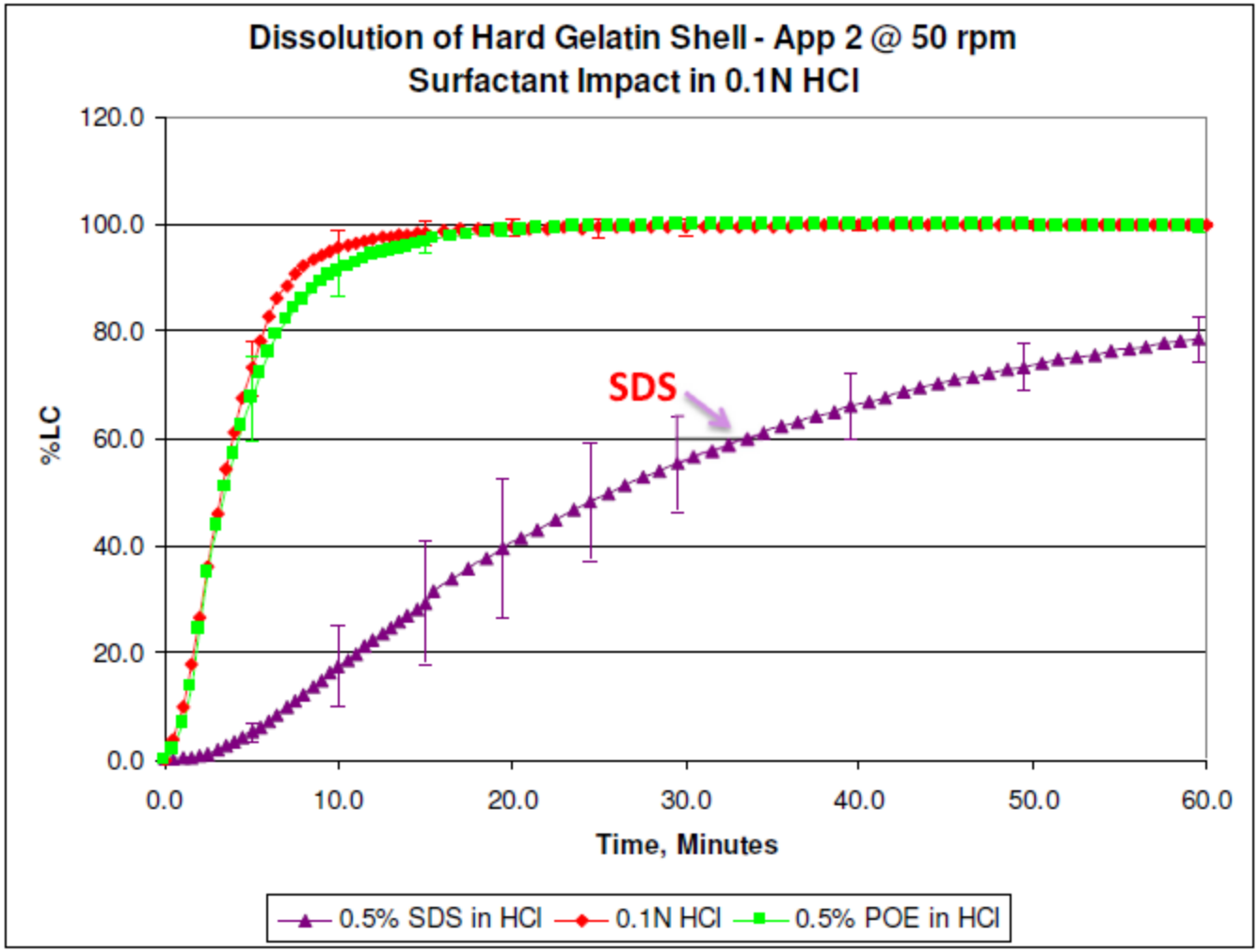
Media Stability

- Raw powders of enzymes are stable for long periods of time at refrigerated conditions (2-8C)
- When enzymes are added to media, stability is greatly reduced
- Media with enzyme should be used within 4 hours of preparation (Jian-Hwa Han, Abbvie)
- Best to prepare and store buffer, and add enzymes when needed on day of use

Pre-Treatment of Surfactants

If surfactants or other ingredients used denature the enzyme, then a pre-treatment step can be used.

- Similar to 2-stage dissolution
- Enzyme amount depends on the volume of pre-treatment step, not final dissolution volume
- Pre-Treatment should be under same dissolution conditions as the rest of the test
- Should not exceed 15 minutes, and pre-treatment time is part of total test time


Dissolution of Hard Gelatin Shell - App 2 @ 50 rpm
Surfactant Impact in 0.1N HCl
%LC
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
Time, Minutes
SDS
0.5% SDS in HCl
0.1N HCl
0.5% POE in HCl

Pre-treatment Options

- Media addition method
- Media changeover method
- Apparatus 3

Media Addition Method

Perform test with media w/o surfactant (media A) at a lower volume.

At end of pre-treatment period, add heated media with surfactant (media B) to end up with total correct volume.

Advantages:

- Single vessel, dosage form doesn't need to be moved

Disadvantages:

- Labor intensive/Time consuming
- Less total units of enzyme in media
- Enzyme in media for all samples, potential issues for LC analysis

Media Changeover Method

Perform Pre-Treatment in Media A.

At end of period, move sample to another bath or completely replace media with media B.

Advantages:

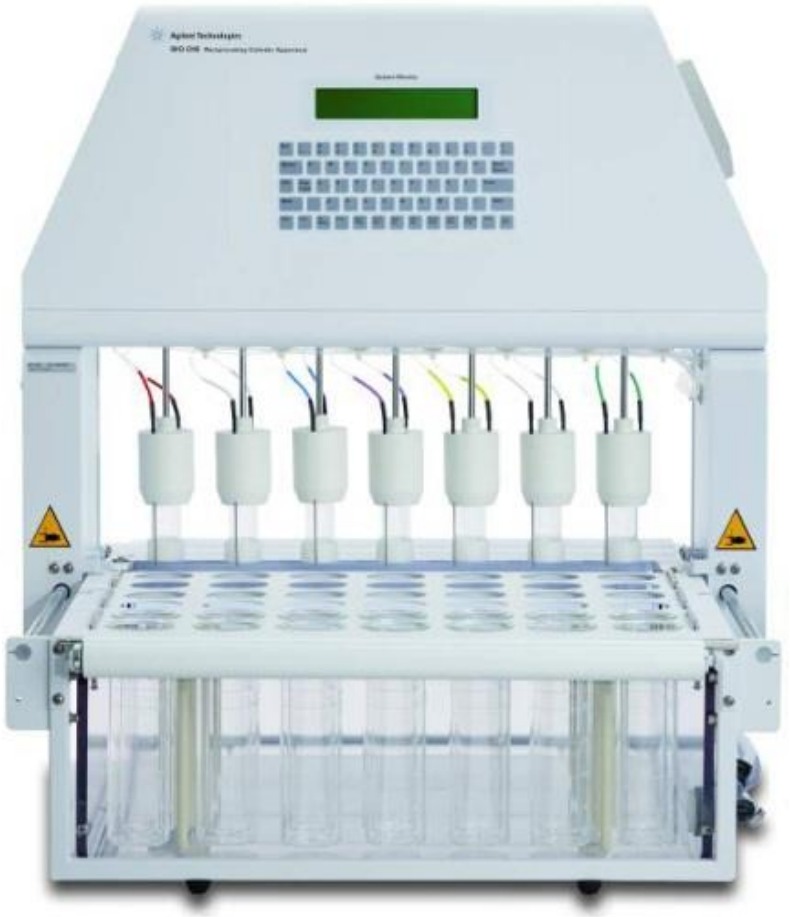
- Less labor intensive?
- Well suited to basket methods
- More units of enzyme

Disadvantages:

- Still labor intensive
- Potential mishandling of dosage form
- May require 2 dissolution units

Bio-Dis Apparatus 3

- Method could be developed as an App 3 method
- If enzymes needed:
  - Perform pre-treatment in row 1 in Media A
  - Row 2 for Media B



Changes to USP <2040> Disintegration and Dissolution of Dietary Supplements

- Similar revisions to USP <711> regarding new enzymes, higher pancreatin, presoaking, etc.
- Chewable tablets now require IR dissolution testing (not related to cross-linking)
- Rupture or Disintegration may be more sensitive test, should be determined on a method to method basis

Failures

What if failure still occurs w/ enzyme?

- If failure is still seen with enzyme, then you fail.
- It is not permissible to add additional enzymes or other techniques with a validated method.

Prevention is Key

- Challenge Product during Method Development
- Proper formulation development
  - Capsule shell selection
  - Excipients
  - Use HPMC instead?
- Proper packaging
  - Humidity
  - Light
  - Free Radicals

Capsule Shell Selection

Much like API, capsule shells vary from vendor to vendor:

- Source of collagen
  - Bovine hides/bones
  - Porcine hides/bones
  - Fish skins (kosher/halal)
  - Recycled leather
- Manufacturing

Excipient Selection

Problem Excipients:

- Corn starch – stabilizer can decompose to formaldedye
- Sugars
- Polyhydric Alcohols
- Oxidating agents
- Sulfated Polysaccharides (chondroitin sulfate)
- Polyethylene glycol (peroxides/aldehydes)
- Metal Ions (colorants/dyes)

API Issues?

If API is causing cross-linking, you may want to consider a HPMC capsule:

- No cross-linking
- Plant derived
- Able to be enteric coated
- Higher cost, fewer vendors
- Limited knowledge compared to gelatin
- APIs w/ carbonyl groups or potential aldehyde generation are prone to crosslinking

Packaging Selection

- Protect from humidity
- Avoid overuse of desiccant
- Protect from light
- Bottles w/ Rayon coilers can release furfural
- Blister packs may be advantageous for sensitive formulations

Cross-Linking

Once it starts, it can't stop.

Summary

- Choice of good capsule shell, packaging, etc. key
- Pre-treatment now an option
- Additional enzymes for mid-pH range
- Challenge method with cross-linked samples
- Important: This is not official! Comments open until January 31, 2015!

References

- USP PF 38(1) – <1094> Liquid-Filled Capsules – Dissolution Testing and Related Quality Attributes
- USP PF 40(6) - <711> Dissolution and <2040> Disintegration and Dissolution of Dietary Supplements
- USP PF 40(6) – Stimuli to the Revision Process: Use of Enzymes in the Dissolution Testing of Gelatin Capsules and Gelatin-Coated Tablets – Revisions
- USP PF 24(5) – Collaborative Development of Two-Tier Dissolution Testing for Gelatin Capsules and Gelatin-Coated Tablets Using Enzyme Containing Media
- USP Workshop of Dissolution Testing of Capsules, March 24-25 2014, Rockville, MD

Special Thanks

Jian-Hwa Han of Abbvie for advice and permission to use graphs