Status: Currently Official on 12-Feb-2025
Official Date: Official as of 01-Dec-2023
Document Type: General Chapter
DocId: GUID-794D7F65-D715-4719-9A14-5C80B55C5FC9_3_en-US
DOI: https://doi.org/10.31003/USPNF_M6181_03_01
DOI Ref: i9urb

⟨1094⟩ CAPSULES—DISSOLUTION TESTING AND RELATED QUALITY ATTRIBUTES

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1. INTRODUCTION

This general information chapter provides approaches for the development of dissolution test procedures for capsules, which are not provided by ▲*Disintegration ⟨701⟩*,▲ (USP 1-Dec-2023) *Dissolution ⟨711⟩*, *Drug Release ⟨724⟩*, *The Dissolution Procedure: Development and Validation ⟨1092⟩*, ▲*Oral Dosage Forms—Performance Tests ⟨1711⟩*,▲ (USP 1-Dec-2023) and *Disintegration and Dissolution of Dietary Supplements ⟨2040⟩*. The chapter also discusses quality attributes associated with capsules that may affect the outcome of dissolution testing.

1.1 Types of Capsules

Capsules can be classified as two main types based on the physical characteristics of the shell: soft capsules and hard capsules. For the purpose of this chapter, soft-shell capsules and hard-shell capsules are referred to as softgels and hardgels, respectively.

▲Softgel capsule is one piece where the manufacture of capsule shell, capsule filling, and final capsule sealing occur in one operation, whereas a hardgel capsule is two pieces (body and cap) where the manufacture of the two pieces and capsule filling occur in two separate operations, very often at different sites.▲ (USP 1-Dec-2023)

Softgels have a thicker shell and typically exhibit a higher degree of elasticity because of the added plasticizer. They also have a slightly longer rupture time when compared with hardgels. By comparison, hardgel capsules have a thinner and more rigid shell than softgel capsules. Both softgels and hardgels are composed of a polymer, e.g., gelatin, starch, ▲pullulan,▲ (USP 1-Dec-2023) or a cellulose derivative such as hypromellose (HPMC), or other polymers, as well as a plasticizer, and water. For hardgels, water acts as the plasticizer, whereas softgels contain high-boiling-point polyols such as glycerol or sorbitol as a plasticizer, and also contain water. Although many parameters affect the physical and chemical properties of the shell, the ratio of polymer to plasticizer primarily determines the rigidity, brittleness, and dissolution performance of the shell.

Capsules can also be characterized by the chemical properties of the fill material (hydrophobic-based versus hydrophilic-based) or by the physical properties of the fill material (solution versus dispersion versus solid). Hydrophobic solutions include neat oils, combinations of miscible oils, or active ingredients dissolved in oil vehicles. Hydrophobic dispersions include active ingredients dispersed or suspended in oil or in oil-wax mixtures. The latter often are termed semisolids. Hydrophilic solutions can be neat liquids, combinations of water-miscible liquids, or active ingredients dissolved in water-miscible vehicles. Hydrophilic dispersions or suspensions include active ingredients dispersed or suspended in hydrophilic vehicles such as polyethylene glycol. Solid fill materials consist of mixtures of excipients and active ingredients whose properties like hydrophilicity/hydrophobicity, polymorphism, particle size, etc., drive the dissolution behavior.

▲Capsules are used for the oral administration of formulations and can be swallowed as a whole unit. Capsules can also be used as a means of measuring a dose of a formulation. One example is in the case of granules, pellets or powders administered with food or beverages, where the capsule is opened and its contents sprinkled over the food or beverage. These capsules can be used when they may be difficult to swallow (e.g., pediatric populations and patients with difficulty swallowing). Another example is capsules that are used in inhalation powder drug products, where the device punctures the capsule to release its content for inhalation by the user. Yet another example is softgels containing liquids or semisolid formulations that have a special design, allowing the user to twist the top of the softgel, then pressing it in order to release its contents for application.▲ (USP 1-Dec-2023)

1.2 Manufacturing and Packaging Issues That Can Affect Dissolution Testing

A number of issues affect the development of a dissolution procedure and the dissolution behavior for capsules, including:

- Properties of capsule shell material
- Properties of the fill material
- Interaction between capsule shell material and fill material

Gelatin is a hygroscopic material, and its moisture content affects the properties of hard and soft gelatin capsules. Since certain excipients are known hygroscopic agents, it is particularly important to monitor the mechanical properties of gelatin capsules stored under various conditions of temperature and relative humidity. The factors that can affect the capsule properties include: moisture exchange

between the shell and the fill material, which potentially can create brittleness in the gelatin shell, and chemical interactions between the fill material and gelatin, which can result in gelatin cross-linking.

The potential for aldehydic impurities and formation of degradants and degradation of the active ingredients or excipients in the formulation should be investigated during product development by means of stability studies (aldehydes contribute to cross-linking; see *2. Cross-Linking in Gelatin Capsules*). Understanding the possible routes of aldehyde or ketone formation, and possible sources of aldehyde, helps to predict capsule behavior. The rate of cooling and drying may modify the characteristics of the active ingredient release from the matrix. The possibility of migration of the active ingredient into the capsule shell, particularly when the softgel is made of gelatin, should be investigated for its impact on dissolution testing.

Capsule shells can become reactive depending on the storage conditions. Product packaging and storage conditions are chosen to prevent adverse effects on capsule quality. The ingress of moisture and/or oxygen into the packaging and through the capsule shell, as well as the rate of ingress, can affect the final shelf life for the product. In some cases, an increase in water content of the capsule shell may produce a measurable reduction in the dissolution time because a moistened product may facilitate polymer hydration.

Due to the water content of gelatin shell (usually between 13% and 16%), this type of capsule behaves as a moisture reservoir which may affect the stability of humidity-sensitive active ingredients.

When a capsule is immersed in an aqueous medium, first the water permeates the walls, then the polymer becomes hydrated, and swells. When fully hydrated, the shell starts to dissolve. The amount of time it takes for water to penetrate capsule shells varies, depending on the nature of the capsule shell and other factors. This time has been reported to be approximately 40 s for gelatin ▲and pullulan▲ (USP 1-Dec-2023) capsules, and about 3 min for HPMC capsules. This delay may be significant only for dissolution testing of immediate-release dosage forms, while it should have minor or no impact on dissolution testing of modified-release formulations.

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2. CROSS-LINKING IN GELATIN CAPSULES

Cross-linking involves the formation of chemical links stronger than the simple hydrogen and ionic bonding between gelatin chains, and affects the thermal reversibility of the sol-gel transition of gelatin in the shell. Cross-linking can be caused by agents present in the capsule fill that react with gelatin molecules, resulting in the formation of a pellicle on the internal surface of the shell. Less often, a pellicle may form on the external surface of the shell arising from reactive agents present in, or derived from the intermediate or final packaging components.

A pellicle is a thin, water-insoluble, clear membrane of cross-linked protein on the inner or outer surface of the capsule that prevents the capsule fill from being released. Cross-linking is evidenced by the observation of a thin membrane or a gelatinous mass during dissolution testing because the pellicle itself may be difficult to observe.

Cross-linking can also be caused by agents or impurities present in the shell, thereby rendering the entire shell matrix insoluble under conditions that normally would dissolve the gelatin shell. One of the strongest and most common types of cross-linking involves the covalent bonding of the amine group of a lysine side chain of one gelatin molecule to a similar amine group on another molecule. This reaction is typically caused by trace amounts of reactive aldehydes. Formaldehyde, glutaraldehyde, glyoxal, and reducing sugars are the most common cross-linking agents. The covalent bonding produced with this type of cross-linking is, for all practical purposes, irreversible, and ▲in vitro▲ (USP 1-Dec-2023) dissolution of the shell must involve the breaking of other bonds, such as the enzyme-mediated breaking of peptide bonds in protein chains.

Gelatin that is chemically modified, e.g., by the addition of succinic acid groups to the lysine side chains, can prevent or at least hinder aldehyde-mediated cross-linking. A weaker type of cross-linking involves complexation of free carboxylic acid groups from two different gelatin molecules with trivalent metal ions such as Fe^{3+} and Al^{3+}. These cations can be found in some of the dyes used as colorants or as low-level contaminants of excipients. For high bloom gelatin (see *6.1.1 Gelatin*), which typically is considered higher quality, cross-linking occurs more readily because fewer links are needed to join greater lengths of gelatin chains.

It is extremely important to know and understand the product formulation to identify possible sources of cross-linking agents and take measures to eliminate or minimize their role in promoting cross-linking. This knowledge can help in the case of post-approval changes in the formulation, and/or packaging material.

Common causes of cross-linking include the following:

- Aldehydes that are present in the active ingredient, excipients, or packaging materials (e.g., in residual solvents), or that may be formed in-situ during storage
- High humidity▲/temperature▲ (USP 1-Dec-2023) (leading to higher oxygen permeability)
- ▲▲ (USP 1-Dec-2023)
- Substances that ▲may decompose to form aldehydes (e.g.,▲ (USP 1-Dec-2023) hexamethylenetetramine ▲in corn starch▲ (USP 1-Dec-2023)), resulting in the formation of ammonia and formaldehyde▲▲ (USP 1-Dec-2023)
- Rayon coilers that contain an aldehyde functional group (furfural)
- Polyethylene glycol may contain peroxides and aldehydes
- UV light, especially with high heat and humidity
- Aldehyde formation promoted by elevated temperatures

Dissolution testing of cross-linked capsules can result in slower release of the drug or no release at all. On rare occasions, if there are defects in the liquid-filled capsule seam, the capsule can rupture at the seam even in the presence of cross-linking in the gelatin, resulting in an early release of the capsule fill in the dissolution medium. The degree of cross-linking is not uniform within one capsule or among different capsules. As consequence, there is a higher variability in the dissolution results if the gelatin capsules are cross-linked. Enzymes can be added to the dissolution medium to overcome this problem (see ⟨711⟩▲and *3.2.3 Use of Enzymes*▲ (USP 1-Dec-2023)). Enzymes should not be used in the absence of such evidence.

▲The evidence of cross-linking can be confirmed visually or experimentally in the following ways:

- Visual observation of a thin membrane (pellicle) or gelatinous mass around the capsule during the dissolution test. Pellicle formation indicates that cross-linking has occurred to an advanced degree. Lower levels of cross-linking which would not result in a pellicle, but perhaps a gelatinous mass, could cause a reduction in the dissolution rate.
- Capsule switching test: the content of the cross-linked capsule is transferred into a fresh shell; subsequently, the emptied cross-linked capsule shell is filled with a fresh blend of the same formulation. The switched capsules are then subjected to dissolution testing. A comparison of the in vitro dissolution results of the cross-linked capsule shell filled with fresh blend versus that of fresh capsules containing the aged formulation blend can confirm whether the dissolution slow down of the aged gelatin capsules is due to cross-linking.
- Dissolution results that show, randomly, a very slow or reduced dissolution rate when compared to the previous data.

Some instrumental analytical techniques have been used to investigate the capsule shell to determine the nature and extent of cross-linking. Examples of such techniques are:

- Ultraviolet/Visible (UV-Vis) spectroscopy—This technique involves making an aqueous solution of the samples, treating with a reagent, and measuring the change in the UV-Vis spectra. This method has failed to be reproducible due to an aqueous solution not being representative of the intact capsule.
- Fourier Transform Infrared (FTIR)—This technique is not reproducible due to the fact that changes in the IR spectrum of gelatin due to cross-linking are very small, which are difficult to reproducibly quantitate.
- Differential Scanning Calorimetry (DSC)—In theory the glass transition temperature of gelatin should increase with increasing degree of cross-linking. The gelatin glass transitions are very weak thermal transitions, which are often masked by thermal transitions of many additives commonly found in gelatin capsules.
- X-ray diffraction—This may be a promising technique for measuring cross-linking because a higher degree of diffraction may indicate a higher level of cross-linking.

[Note—Precise quantitation of cross-linking is difficult and unlikely to be achieved using these methods; however, a relative comparison may be possible.]▲ (USP 1-Dec-2023)

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3. DISSOLUTION PROCEDURE DEVELOPMENT

Design of a procedure for dissolution testing of capsules depends on the product formulation. The composition of the fill, the solubility of the active ingredient(s) in the fill, and the dispersion of the fill into the dissolution media all have an effect on the dissolution behavior of a given fill formula. For example, a formulation that contains a lipid-soluble active ingredient with hydrophobic excipients and a melting point in excess of 37° will likely not release the active ingredient into solution in aqueous media in a timeframe that is consistent with the expectations for immediate-release dosage forms. By contrast, a freely water-miscible active ingredient dissolved or dispersed in a water-soluble or water-dispersible fill formula at room temperature will be released into solution very soon after the shell ruptures and the dosage form releases the fill material into the media.

The specific design of the formulation and the target release profile should be known when developing a dissolution method for any given product. For lipid-based formulations that often are less dense than aqueous media, the release of the fill from the capsule may be driven by buoyancy. If the formulation has been designed to be self-emulsifying or self-microemulsifying, the formulation will efficiently disperse into most aqueous media. Formulations such as those that consist simply of a triglyceride with no additional co-solvent or emulsifier will rapidly float to the dissolution medium surface in the vessel. In this situation, the quantitative results from a dissolution test provide the rate of drug partition from this floating layer. The apparatus selection is perhaps the most critical step for these types of capsules because the efficiency with which the capsule contents mix with dissolution media is highly influenced by the hydrodynamics of the agitation. Once the apparatus has been selected, other variables to consider in developing a dissolution test are rotation speed, dip rate or flow rate, surfactant/solubility enhancer type and concentration, medium volume▲, mesh size of the basket or screens, media deaeration, sinkers,▲ (USP 1-Dec-2023) and pH (see ⟨711⟩, ⟨1092⟩, and ⟨2040⟩).

To better guide dosage form design, it may be useful to consider other characteristics of the product such as solubility of the active ingredient(s) in different media ▲(see *Solubility Measurements* ⟨1236⟩)▲ (USP 1-Dec-2023) , intrinsic dissolution of the active ingredient(s) (see *Intrinsic* ▲*Dissolution—*▲*(USP 1-Dec-2023)* *Dissolution Testing Procedures for Rotating Disk and Stationary Disk* ⟨1087⟩), dispersibility, globule size for emulsions, micelle formation, digestion, precipitation on dilution in media, phase behavior studies, and burst tests to detect gelatin cross-linking.

The rupture test may be a useful tool in the early steps of the development and evaluation of the formulation.▲Rupture occurs when the capsule shell is breached, exposing the fill contents. Typically, the rupture test is used only in the early stages of development. Later, it is replaced by a dissolution or a disintegration test, with appropriate justification.

The use of enzymes to address cross-linking is considered a second method. Special considerations in method development with the addition of enzymes is addressed in *3.2.3. Use of Enzymes*.▲ (USP 1-Dec-2023)

3.1 Apparatus

As is the case with other oral solid dosage forms, USP *Apparatus 1* (basket), USP *Apparatus 2* (paddles), USP *Apparatus 3* (reciprocating cylinder), and USP *Apparatus 4* (flow-through cell) are most often chosen as the dissolution apparatus for capsules (see ⟨711⟩ and ⟨2040⟩). However, several specific ▲physical and chemical properties and characteristics▲ (USP 1-Dec-2023) must be considered in selecting and using a dissolution apparatus for capsules:

- Capsules may be filled with a material that has a specific gravity less than that of water. Therefore, the capsules may float in an aqueous dissolution medium ▲and consequently must be submerged via the basket or with the aid of a sinker when using the paddle apparatus.▲ (USP 1-Dec-2023)
- Instead of dissolving completely during the dissolution test, the capsule shell may soften and disintegrate into a sticky or waxy mass that can adhere to any point in the dissolution vessel ▲, basket, or the underside of the basket shaft holding the basket, which may generate▲ (USP 1-Dec-2023) high variability in the results.
- ▲During the course of the test,▲ (USP 1-Dec-2023) the capsule fill material may form ▲an oily layer on the surface of the dissolution medium. This may require the use of surfactants to solubilize the fill material in the aqueous dissolution media.▲ (USP 1-Dec-2023)

The capsule dissolution process involves three stages: 1) rupture of the capsule shell, 2) release and dispersion of the capsule fill material, and 3) dissolution of the active ingredient(s) in the medium. Each dissolution apparatus referred to previously can achieve these three stages, but they may cause different hydrodynamic effects upon capsules as on any other dosage form as well.

3.1.1 USP APPARATUS 1 (BASKET)

This apparatus has the advantage of enclosing the capsules, preventing them from floating freely in the medium. For certain capsules, however, baskets may not be suitable. As the capsule ruptures, the material from the capsule shell may clog the basket's mesh, and for hydrophobic fill materials the oil phase released from the capsule may not disperse into fine enough droplets in the basket to efficiently pass through the mesh. A larger-size mesh may be needed to overcome these limitations.

3.1.2 USP APPARATUS 2 (PADDLES)

This apparatus does not ▲▲ (USP 1-Dec-2023) prevent the capsules ▲or contents▲ (USP 1-Dec-2023) from floating. In these instances, wire coils can be wound around the capsules, or commercially available sinkers can be used to encase the capsules and hold them on the bottom of the vessel, allowing the fill to become exposed to more of the medium (see, ⟨1092⟩, *1.4 Choosing an Apparatus*, *2.2 Sinkers*, *2.3 Agitation*). Sinkers can also be used to prevent the capsule from sticking to the vessel walls and to provide better contact with the dissolution medium, even if the capsule does not float and remains at the bottom of the vessel. The shape and size of the sinker can play an important role in the dissolution profile and should be selected carefully. The swelling that occurs when the capsule is placed in contact with the dissolution medium should be considered when defining the size of the sinker to be used.

3.1.3 USP APPARATUS 3 (RECIPROCATING CYLINDER)

This apparatus, like *Apparatus 1*, encloses the capsules. The mesh at the bottom of the cylinder, however, may become clogged with undissolved shell. Changing the mesh size may alleviate this problem. The different mechanism of agitation of *Apparatus 3* provides very different hydrodynamics compared with *Apparatus 1* and *2*. This characteristic may assist in dispersing hydrophobic droplets, avoiding the formation of layers, and some problems caused by the buoyancy of the capsule. In the case of *Apparatus 3*, a change to different media during the course of the dissolution experiment is possible. This allows dissolution profiles to be determined at different pH values, which is useful for targeted-action or modified-release dosage forms. For cases where the active ingredient has been dispersed but not dissolved, this apparatus may not be a good choice since significant sample loss will occur when moving the cylinder from one ▲row▲ (USP 1-Dec-2023) to the other. However, USP *Apparatus 3* has a tendency to generate foam when surfactants are added to the media. In this situation, an appropriate antifoaming agent may be added to the medium.

An alternative to *Apparatus 3* is the disintegration apparatus described in ⟨701⟩. The use of disks should be avoided because of clogging. If disks are not used, the capsule will float and may not be uniformly wetted. This apparatus offers more turbulent conditions that could be useful in the dispersion of hydrophobic filling, avoiding the formation of layers and counteracting the buoyancy of the capsule.

3.1.4 USP APPARATUS 4 (FLOW-THROUGH CELL)

This apparatus also encloses the capsules and has a filter. Several types of cells are available depending upon the application. The use of the flow-through cell designed for lipid-filled softgels may be useful for certain types of formulations. ▲▲ (USP 1-Dec-2023) Use of *Apparatus 4* also makes it possible to change to different media and to alter the flow during the course of the dissolution experiment. In addition, this

equipment can be set up for low or high volumes of media that can be used with low-strength products and with poorly soluble active ingredient(s), respectively.

Other apparatus may be considered with proper justification, with each candidate apparatus evaluated for variations in media composition, media volume, agitation or flow rate, and other test parameters to determine what effect each has on the dissolution performance of the product.

3.2 Medium

General recommendations for the selection of appropriate dissolution media can be found in chapters ⟨1092⟩ and ⟨2040⟩, FDA Guidance for Industry *Dissolution Testing of Immediate-Release Solid Oral Dosage Forms*, ▲FDA Guidance for Industry *Dissolution Testing and Acceptance Criteria for Immediate-Release Solid Oral Dosage Form Drug Products Containing High Solubility Drug Substances*,▲ (USP 1-Dec-2023) and FDA Guidance for Industry *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In vitro/In vivo Correlations*.

Characteristics of the dissolution medium that may affect the opening or rupture of the capsule shell, the release and dispersion of the capsule fill material, and the dissolution of the active ingredient(s) are discussed in the following sections.

3.2.1 ACHIEVING SINK CONDITIONS

As in the development of any effective dissolution procedure, the medium preferably, but not necessarily, should provide sink conditions for the active ingredient(s) in an environment that ensures suitable stability and, preferably, is physiologically relevant for the product (see ⟨1092⟩).

If the fill is water-soluble or at least readily dispersible in an aqueous medium, and if the active ingredient itself is also soluble, sink conditions may be achieved in a manner comparable to that for any other solid oral dosage form. Liquid-filled capsules, however, may contain either a matrix or an active ingredient (or both) that are hydrophobic or water-insoluble. In this case, a medium with surfactants may be needed (see below). The use of organic co-solvents to improve sink conditions is discouraged and should be employed only as a last resort and at a minimum amount with appropriate justification. In practice, the presence of organic solvents in the medium may inhibit the dissolution of the shell.

▲▲ (USP 1-Dec-2023)

To establish a suitable medium, several different dissolution media should be evaluated to identify the one that achieves appropriate sink conditions with the lowest quantity of solubilizing/dispersing agent. The effects of pH, ionic strength, buffer counter-ion, and/or co-solvent on active ingredient solubility and enzyme activity, particularly for hydrophobic active ingredients, in addition to the relative partitioning of the active ingredient between the matrix and medium, must also be evaluated.

3.2.2 USE OF SURFACTANTS/DISPERSING AGENTS/SOLUBILITY ENHANCERS

Surfactants, dispersing agents, or solubility enhancers may be used in the dissolution medium when the capsule fill and/or the active ingredient are hydrophobic or water-insoluble. They may also be used if the media described in ⟨1092⟩ and in the FDA guidances are ineffective in dispersing the capsule fill or in achieving proper sink conditions for the active ingredient.

Surfactants, dispersing agents, and solubility enhancers include:

- Anionic:
  - Sodium dodecyl sulfate (SDS; or sodium lauryl sulfate, SLS)
  - Bile salts (sodium deoxycholate, sodium cholate)
- Cationic:
  - Cetyltrimethylammonium bromide (CTAB)
  - Hexadecyltrimethylammonium bromide (HTAB)
  - Methylbenzethonium chloride (Hyamine)
- Nonionic:
  - Polysorbates (Tween)
  - Polyoxyethylene sorbitan esters
  - Octoxynol (Triton X100)
  - *N,N*-dimethyldodecylamine-*N*-oxide
  - Brij 721
  - Polyoxyl castor oil (Cremophor)
  - Nonylphenol ethoxylate (Tergitol)
  - Cyclodextrins
  - Polyoxyl 10 lauryl ether
- Zwitterionic:
  - Lauryl dimethyl amine oxide (dodecyldimethylamine oxide, DDAO)
  - Lecithin

Although useful as solubilizing agents, surfactants should be used cautiously because they can interact with the gelatin in the capsule shell and can hinder disintegration or dissolution. They can also inhibit enzymes that may be used to hydrolyze the gelatin shell and/or the

fill.

One of the most common anionic surfactants used for dissolution testing of tablets, SLS, exhibits both of these adverse effects, particularly at lower pH (e.g., in simulated gastric fluid). In addition, SLS forms insoluble precipitates in the presence of potassium ions. SLS is one of the surfactants less compatible with enzymes. Only a very high quality grade of SLS should be employed in dissolution media because of the potential interference of its impurities in the quantitation step in dissolution testing. Therefore, the use of SLS should be considered only in the absence of other alternatives.

Other anionic or cationic surfactants have also been shown to affect gelatin solubility, and the extent of these interactions should be considered in the selection of the dissolution medium. Cationic surfactants should be avoided in formulations that contain fatty acids, because the combination potentially forms insoluble precipitates.

▲In addition to dispersing/dissolving the matrix and active ingredient(s), the medium must neither interfere with the activity of any enzyme used nor negatively interact with the capsule shell or formulation. Developing a suitable medium for hydrophobic systems therefore may require considerable experimentation.▲ (USP 1-Dec-2023)

3.2.3 USE OF ENZYMES

Proteolytic enzymes ▲▲ (USP 1-Dec-2023) may be used when ▲there is evidence of the presence of cross-linking in▲ (USP 1-Dec-2023) the gelatin capsule shell ▲(see *2. Cross-linking in Gelatin Capsules* and ⟨711⟩).▲ (USP 1-Dec-2023) Pancreatin▲, an example of a proteolytic enzyme,▲ (USP 1-Dec-2023) has the advantage of also possessing lipase activity, which makes it useful when the capsule fill material is a triglyceride.

Tests should be carried out to ensure that the enzymes used in the dissolution testing do not adversely interact with the formulation. ▲Enzymes have been reported to increase the risk of hydrolysis of substances which have an amide bond.▲ (USP 1-Dec-2023) The dissolution medium should also be optimized (e.g., by adjusting the pH, ionic strength, etc.) so that it preserves enzymatic activity while maintaining sink conditions for the active ingredient.

▲**Enzyme Activity:** The actual enzyme activity value is critical to the efficiency of the function of digesting the gelatin in the cross-linked capsule shells.

Enzyme activity is heavily dependent on the substrate being used to measure it. The protease activity may be different depending on the type of substrate being used. The protease activity must be determined by the procedure stated under the respective reagent specification in the Reagent Specifications section of USP for pepsin and bromelain or the appropriate *USP* monograph for *Pancreatin* and *Papain*.

Dissolution Testing: The dissolution testing of the capsules should be conducted following the instructions in ⟨711⟩. In the presence of cross-linking in the gelatin capsules, the test should be repeated. New dissolution medium should be prepared with the appropriate enzyme in the recommended amount, and the dissolution testing should be done with new capsules following the instructions in ⟨711⟩.

Dissolution Testing During Stability Studies: If the capsules were stored according to the conditions stated in the *USP* monographs for capsule shells and the formulation development was done considering the possible sources of cross-linking, typically, cross-linking in gelatin capsules is going to be found during stability studies. The dissolution testing during stability studies should start with the dissolution medium stated in the original method without the enzyme. When evidence of cross-linking is found, the test should be done with the addition of the appropriate enzyme to the dissolution medium. As cross-linking does not stop even if the agent causing it is removed, from this time point forward, the dissolution testing of stability samples can be carried out directly with the addition of the appropriate enzyme to the dissolution medium.

Surfactants or Other Medium Components That May Denature Enzymes: Surfactants and other medium components may denature the enzyme. If such ingredients are part of the dissolution medium and if enzymes are going to be used to digest the cross-linked gelatin, a special step should be included in the final dissolution method to preserve the protease activity of the enzyme. This procedure is commonly known as Tier 2, with Tier 1 being the original dissolution procedure without the enzyme.

Tier 2 Dissolution Testing: Typically, the Tier 2 dissolution testing will have two dissolution media: **Medium A** and **Medium B**.

Medium A contains the appropriate enzyme in the solvent of the original dissolution medium (e.g., water, acid, or buffer solution) in the amount stated in ⟨711⟩, in a smaller volume of dissolution medium than the one stated in the original method. The enzyme is going to digest the cross-linked gelatin, allowing the release of the capsule filling to the medium. Typically, the duration of the pre-treatment step is not more than 15 min. All the other conditions of the test (apparatus, rotation, or flow rate) should remain as described in the method or monograph.

Medium B is a solution in the appropriate solvent (e.g., water, acid, or buffer solution) with the surfactant or the component that may denature the enzyme in a concentration that when mixed with *Medium A* is going to reach the final concentration stated in the original dissolution procedure. The dissolution test is going to be run for additional time, with all the other test conditions (apparatus, rotation, or flow rate) as described in the method or monograph, to reach the total time specified in the method or monograph. As an example, if the total time of the dissolution test is 45 min, the samples will be in contact with *Medium A* for 15 min and, after the addition of *Medium B* to the apparatus, for 30 more min. The volume of *Medium A* and *Medium B* should be selected in a case-by-case approach, with *Medium A* volume being smaller than the volume stated in the original method or monograph. The volume of *Medium B* should be such that it will reach the volume stated in the original method or monograph after its addition to *Medium A*. Any deviations from these recommendations above need to be scientifically justified.

The first example of Tier 2 testing is located within the *USP* monograph for *Acitretin Capsules, Performance Tests, Dissolution Test 2*, where in *Tier 2* the volume of *Medium A* and *Medium B* is 450 mL each, resulting in the original medium volume of 900 mL. The second

example of Tier 2 testing is located within the *USP* monograph for *Ziprasidone Capsules, Performance Tests, Dissolution Test 2*, where in *Tier 2* the volume of *Medium A* is 700 mL, and *Medium B* is 200 mL, resulting in the original volume of 900 mL.

Forced Cross-Linking: During product and dissolution method development, forced formation of cross-linking in the gelatin capsule may be useful to establish the type and amount of enzyme that will be used in the test, and to better understand the behavior of the formulation in the dissolution medium. This can be achieved by spiking excipients with known amounts of formaldehyde or other cross-linking agents, or exposing the capsules to formaldehyde or high humidity and high temperature for an extended period of time. One possible procedure to generate cross-linking in the gelatin capsules is the one described in Gold TB et al.[1] Other procedures can be found in the literature.▲ (USP 1-Dec-2023)

3.2.4 pH

In addition to establishing a drug pH-solubility profile during the dissolution development, the following specific issues should also be evaluated:

- The effect of media pH on the swelling or dissolution of the capsule based on the type of gelatin used in the product: either Type A (for which the medium pH typically is ▲7.0–9.0▲ (USP 1-Dec-2023)) or Type B (for which the medium pH typically is 4.7–5.4)
- The need for enzymes may influence the selection of a specific pH range in order to be adequate for the active ingredient solubility and stability, and to minimize effects on enzyme activity

▲▲ (USP 1-Dec-2023)

3.3 Sampling

Establishing the proper sampling technique and location for capsules follows the procedures described in chapters ⟨711⟩ and ⟨1092⟩. In the case of hydrophobic liquid filled capsules, where the fill material typically forms a film on the surface of the dissolution medium during the course of the test, sampling must be performed in such a way that the cannula penetrates the oily layer without becoming clogged. In addition to standard validation and compatibility studies, care must be taken when a filter is used to ensure that it does not become clogged with oil or undissolved capsule shell material when the sample is taken. Similar considerations apply to automated sampling equipment because filters and transfer lines may become obstructed during sampling. To address this issue, the use of surfactants and/or enzymes in dissolution media may be needed to better solubilize the capsule shell and fill. Another point to be considered in the case of automated sampling equipment is the probe. If it remains inside the medium during the test, it could perturb the oily layer and possibly influence the hydrodynamics, thus changing the dissolution profile. An alternative approach is to remove the probe and to introduce it just at the time of the sampling. The sampling method should be validated for each product.

3.4 Quantitation

Like dissolution samples from other oral solid dosage forms, dissolution samples from capsules can be quantitated using chromatographic, spectrophotometric, tandem chromatography-mass spectrometry, and other techniques after adequate method development and validation (see ⟨1092⟩ and where special considerations are described under *4. Method Validation*).

Change to read:

4. METHOD VALIDATION

In addition to the general method validation parameters discussed in ⟨1092⟩, the following performance characteristics may be evaluated:

- Effect of pH and ionic strength on drug solubility and on enzyme activity, if enzymes are used.
- For hydrophobic active ingredient(s), the relative partitioning of the active ingredient(s) between the matrix and the medium, as well as the potential for an adverse effect on release into the aqueous medium.
- For chromatographic procedures, the potential adverse effect of the surfactant, if used, on the chromatographic separation; as part of the robustness study, evaluation of different concentrations of surfactant, different surfactant types, interaction with buffer salts, etc.
- For spectrophotometric procedures, absorbance from the potential contribution of the capsule shell should be evaluated.
- Potential adverse effects of the surfactant or enzyme, if present, on the lifetime of the chromatographic column.
- Qualification of the sampling procedure to prevent clogging of the cannula, transfer tubing, or filters.
- Validation of the ▲dissolution method step with enzyme (Tier 2) and of the pre-treatment step when the dissolution medium contains components that may denature the enzyme.
- Verification that the conditions of the Tier 2 step do not affect the dissolution profile when compared to the Tier 1 test. This may be accomplished by performing parallel analysis with the drug formulation filled in non-cross-linked capsules run in the required dissolution media (Tier 1) and the same media with the enzyme added (Tier 2). The resulting dissolution profiles should be the same, showing that the enzyme did not adversely affect the drug release profile.
- Verification that the quantitative procedure in the Tier 2 step is specific, accurate, precise, linear, and has solution stability.

4.1 Cleaning Considerations

Cleaning methods for dissolution apparatus components (e.g., vessels and stirring elements) and for automated systems, if employed, should be developed and qualified for their effectiveness in removing traces of residue from product or media from previous tests. Interference from residual active ingredient(s), excipients, and media (including surfactants) should be mitigated to ensure the integrity of

each subsequent dissolution test. While established glassware cleaning procedures may document best practices for cleaning laboratory glassware, more stringent procedures may be needed for dissolution equipment, especially automated equipment, to ensure that run-to-run cleaning procedures are effective and that in-run procedures, which adequately prime and purge sampling probes, inline filters, tubing, pumps, and dispensing systems, were effective in avoiding carryover from previous tests or dilution from rinse solutions from each successive sample collected during an automated sampling sequence for the dissolution test.▲ (USP 1-Dec-2023)

Change to read:

5. SUGGESTIONS FOR STARTING POINTS

Based on the considerations discussed above, possible starting points for establishing a dissolution test for capsules based on the solubility characteristics of the fill and the active ingredient(s) are the following:

Possible formulations:

1. Hydrophilic fill/active ingredient is soluble in aqueous media
2. Hydrophobic fill/active ingredient is poorly soluble in aqueous media
3. Hydrophilic fill/active ingredient is poorly soluble in aqueous media
4. Hydrophobic fill/active ingredient is soluble in aqueous media

5.1 Medium

Evaluate the solubility of the active ingredient(s) in aqueous media within a pH range of 1.0 to 7.2–7.5.

5.2 Medium Additives

Enzymes ▲▲ (USP 1-Dec-2023) may be used if the product shows evidence of cross-linking in the gelatin capsule. ▲See *3.2.3 Use of Enzymes*.▲ (USP 1-Dec-2023) If the product is a formulation such as *2* or *3* above, evaluate the use of surfactants and possible interaction with enzymes.

5.3 Apparatus

USP *Apparatus 1* or *2*. If the capsule is filled with low specific gravity liquid and has a tendency to float, sinkers should be used. If the product is a formulation such as *2* or *4* above, consider *Apparatus 3* as an option.

5.4 Agitation Speed

Agitation speed typically is 50–100 rpm for baskets and 50–75 rpm for paddles. Higher speeds should be justified.

5.5 Time Points

A dissolution profile should be established during the development phase to identify when the rate of active ingredient release has leveled off.

▲5.6 In Vitro/In Vivo Correlations

An in vitro/in vivo correlation (IVIVC) is possible with capsules, as with any solid oral dosage form. See *In Vitro and In Vivo Evaluation of Oral Dosage Forms* ⟨1088⟩. However, if cross-linking becomes an issue with an IVIVC, the Tier 2 procedure with enzyme would have to be validated to show that the correlation still presents with the new media.▲ (USP 1-Dec-2023)

Change to read:

6. CRITICAL QUALITY ATTRIBUTES

6.1 Shell Composition

Knowledge of the composition of the shell (polymer, plasticizers, water content, etc.), as discussed at the beginning of this chapter, is an important aspect and helps determine critical quality attributes. Other important aspects of understanding the critical quality attributes are the composition and properties of the gel mass and finished shell. In its simplest form, the shell of a capsule is prepared from a molten gel mass, which in the case of hardgels, is comprised of gelatin or other polymers and water, and in the case of softgels, the shell is comprised of gelatin and a plasticizer dissolved in an aqueous vehicle. The ratio of polymer to plasticizer varies depending on the desired performance traits of the shell, the size of the shell, and the composition of the fill material. Other minor components added to the gel mass may include colorants, flavors, stabilizers, buffers, and opacifiers. The physical characteristics and quality of these minor components are central to the design of high-quality and robust formulations. The composition of hardgels usually does not vary with capsule size.

6.1.1 GELATIN

Gelatin capsule manufacturing process: For hardgels, there are two separate manufacturing steps: 1) manufacturing the empty shells, and 2) the filling process. Capsule shells are produced, packaged, and shipped to the dosage form manufacturers. Capsules are then filled and sealed. Most modern capsule-filling machines are designed to allow accurate filling of powders, granules, pellets, tablets, and combinations of these can be modified to allow hot or cold liquid to be filled into hardgels. An essential part of a liquid-filling operation is the ability to effectively seal the capsule. This sealing process is a critical process parameter, and detailed knowledge of all the aspects of this process is important.

For softgels, the formation of the capsule, the filling process (i.e., encapsulation process), and sealing of the capsules occur simultaneously. During the encapsulation process, it is important to monitor the shell thickness, seam quality, capsule weight, and the fill weight using statistical process controls.

Chemistry of gelatin: Understanding the chemical nature of gelatin is another important component of the critical attributes of the formulation. Gelatin is graded primarily on the strength of the gel. Depending on the process and the tissue source, noticeable differences in ▲bloom▲ (USP 1-Dec-2023) strength are apparent among suppliers and even between lots from the same supplier. Consequently, controlling the strength of the gelatin from batch to batch, measured as bloom strength, is key to obtaining a consistently performing product. Bloom strength is a measure of the strength of the gel prepared at a set concentration of gelatin in water under controlled conditions, and is a function of the molecular weight of the gelatin, the concentration of the gelatin in the gel, and the pH of the gel. It is a measure of the resultant gel's resistance to compression and is reported in bloom-grams or grams. Bloom strength increases when the gelatin concentration in the gel increases, when the average molecular weight of the gelatin increases, and when the pH of the gel approaches neutrality (from either direction). In addition, as bloom strength increases, the cost of gelatin increases and the rate of gel dissolution decreases. Bloom strength also has an effect on the clarity and color of liquid-filled capsules. For gelatin with higher bloom strength, less gelatin is needed to produce a suitable shell, which results in a clearer shell and a reduced need for colorants and dyes in order to produce the desired hue. Although gelatin can be purchased with bloom strengths ranging from 50 to 300, most gelatins used in the manufacture of liquid-filled capsules have a bloom strength of about 150–200 for softgels and 220–280 for hardgels. Gelatin manufacturers commonly blend different sublots of gelatin to meet bloom requirements. These important characteristics of gelatin must be taken into account when characterizing the product under development.

6.1.2 CHEMISTRY OF OTHER POLYMERS

Suitable consideration should be given to the other polymers used to manufacture capsule shells. Iota and kappa carrageenans, modified corn, potato, and pea starches, and modified celluloses, together with plasticizers are also used for the preparation of capsule shells. Carrageenans are polysaccharides extracted from sea weeds. They possess the required gelling characteristics similar to gelatin. However, kappa carrageenan produces a brittle gel, and iota carrageenan a soft and elastic gel. Carrageenans are usually combined with modified starches and plasticizers to form the capsule shell. Modified celluloses, such as HPMC, ▲and other polymers such as pullulan▲ (USP 1-Dec-2023) are also used for the manufacturing of hardgels. Compared to gelatin shell, the shell prepared using the polymers mentioned above will have some advantages, such as non-crosslinking, being able to handle wider pH ranges, and tolerance of high fill temperatures.

6.1.3 PROCESSING AIDS

Processing aids include gelatin ribbon lubricants in the softgel manufacturing process. Commonly used ribbon lubricants include mineral oil and medium chain triglyceride. Since ribbon lubricants are applied to both the inner and outer surfaces of the capsule shell, the evaluation of their potential adverse effects is focused on the capsule itself and primarily on gelatin cross-linkage. ▲SLS is used as a processing aid for hard gelatin capsules to improve glide properties of capsules during the filling process at the dosage form manufacturers.▲ (USP 1-Dec-2023)

6.2 Stability and Storage Conditions

Knowledge of the stability and reactivity of gelatin or other polymers is essential to anticipate their influence on final product quality. At room temperature, the capsule shells as supplied are relatively effective in protecting the fill from oxygen and its effects, and when an opacifier such as titanium dioxide is added to the shell, it can prevent photodegradation of the fill. In addition, the low water activity of the shell does not promote microbial growth. For bacteria, yeasts, and molds to grow, a higher water activity of at least ▲0.80▲ (USP 1-Dec-2023) a_w is required; the water activity of capsule shells typically is less than 0.40 a_w.

Storage conditions of the final product range from refrigeration to standard room temperature. Products should be stored according to the label directions. Brief excursions outside of these conditions should be evaluated to determine their influence on the final product quality. Storage conditions and duration for the empty capsule shells should also be considered. ▲See *USP* monographs for Hard Gelatin Capsule Shells, Hard Hypromellose Capsule Shells, and Hard Pullulan Capsule Shells.▲ (USP 1-Dec-2023)

6.3 ▲Brittleness

The strength and durability of hydrophilic polymer films can be affected by their moisture content which, in turn, will be affected by changes in the relative humidity of the immediate environment. This applies to hardgel capsule shells since they are, in essence, hydrophilic polymer films. Hardgel capsule shells are designed with tight tolerances, and a change in moisture content of the capsule shell can impact capsule filling. If the relative humidity around the capsules increases, the capsule shells will absorb moisture and can swell, thereby changing their dimensions. If the relative humidity drops, then the capsule shells will lose moisture and become brittle, which may also impact capsule fill operations. This phenomenon is more problematic with hard gelatin and hard pullulan capsules than with hypromellose capsules. Thus, it is important to store empty capsule shells in moisture-tight packaging and under controlled room temperature. Embrittlement can also occur in storage with hard gelatin capsules containing hygroscopic fills, since such materials will draw water out of the shell. In general, if the fill has a water activity of <0.2 a_w, water will be lost from gelatin hard capsule shells and embrittlement may occur.

For capsules used in inhalation devices, both embrittlement and softening due to moisture absorption can impact the safety and efficacy of the drug product. Brittle capsules may break rather than be punctured and release gelatin particles on piercing. Softer capsules may not

puncture properly, thus interfering with the release of its contents.

There are several methods for the assessment of capsule brittleness; however, there is no universally accepted test. Users of hardgel capsules should confirm with their suppliers as to the testing details if capsule brittleness is a concern.

6.4 Overall Potential Capsule Defect Assessment

During the capsule manufacturing process, the following defects may occur:

- Critical Defect—Affects the ability of the capsule to function as a dosage form delivery system, or contributes to major processing issues during filling operations (i.e., holes, split, mashed, etc.)
- Major Defect—Has the potential to cause a processing problem during capsule filling operations (i.e., dents, short cap, long body, etc.)
- Minor Defect—Has no effect on a capsule's performance as a whole or on the capsule filling operation, but detracts from the capsule's cosmetic appearance (i.e., bubbles, strings, crimps, etc.)▲ (USP 1-Dec-2023)

6.5 Formulation Development and Manufacturing for Liquid-Filled Capsules

It is important to understand the properties of the active ingredient(s) during formulation development.

Formulations developed using liquid-filling technology may be applicable when the active ingredients display 1) poor solubility in aqueous systems, 2) short half-life requiring frequent dosing, 3) low melting point, 4) low dose/high potency where containment is important, 5) requirement for taste or odor masking, and 6) critical chemical or physical stability as in the case of highly hygroscopic active ingredients. Other factors to consider during formulation development include:

- Compatibility and stability of excipients and active ingredient(s) over time.
- Temperature-dependent solubility of the active ingredient(s) in the lipid.
- Aging/polymorphic characteristics of the lipid.
- Adequate characterization of the saturation/supersaturation status of the active ingredient(s) in the lipid formulation in order to avoid precipitation of the active ingredient(s). Optimally, saturation status should be determined when the formulation is at equilibrium with the shell.
- Stability in solution under stress conditions.
- Aldehyde formation and degradation of active ingredient(s).
- Influence, if any, of the rate of cooling on the structure of certain excipients, which may modify the release characteristics of the active ingredient(s).

Important factors to consider during a liquid-filling operation are temperature and viscosity of the fill material and, in the case of a dispersion or suspension, the particle size of the dispersed active ingredient(s). In principle, any excipient found to be compatible with the shell can be used, but in a manufacturing environment the viscosity of the fill material is important. ▲For hardgel capsule filling,▲ (USP 1-Dec-2023) excipients that are solid at room temperature but melt at temperatures up to 70° may be suitable (depending on the shell polymer) for formulating active ingredient(s), provided those excipients yield the desired in vivo performance.

Both shell and fill excipients should be controlled for levels of known cross-linking agents such as formaldehyde and reducing sugars. Imperfections in the shell and/or in the seam may affect dissolution. They may also give rise to leaking of the capsules contents.

The appropriate in-process controls should be in place to monitor and reduce the lot-to-lot variability.

[1] Gold TB, Buice RG, Lodder RA, Digenis GA. Determination of extent of formaldehyde-induced cross-linking in hard gelatin capsules by near-infrared spectrophotometry. *Pharm. Res.* 1997, 14(8): 1046-50. doi: 10.1023/a:1012105412735.

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Pharmacopeial Forum: Volume No. 48(4)

Current DocID: GUID-794D7F65-D715-4719-9A14-5C80B55C5FC9_3_en-US
DOI: https://doi.org/10.31003/USPNF_M6181_03_01
DOI ref: i9urb