An Overview of Dissolution Method Development and Validation for Semisolid Dosage Forms

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BACKGROUND

When most of us think of drug product dissolution testing, images of solid oral dosage forms like capsules and tablets using Apparatus 1 or 2 come to mind. But other dosage forms are also characterized by dissolution, including semisolid forms often used for topical drug delivery. It's worthwhile to look at some of the things specific to those forms in dissolution testing.

Dissolution testing of solid oral dosage forms has been the dominant United States Pharmacopeia (USP) dissolution test for more than four decades. Dissolution is used to understand the effect of changes to the physical characteristics of drug substances and drug products throughout the drug development life-cycle. Dissolution can be used:

- to correlate product safety and efficacy[1]
- to correlate in-vitro test results to in-vivo drug action
- as a routine test for product release and stability studies[2,3]

In August of 2013, mandatory performance testing for semisolid drug products was included in the USP as general chapter <1724> SEMISOLID DRUG PRODUCTS—Performance Tests.[4] This white paper offers an overview of in-vitro performance testing for semisolid drug products, such as creams, ointments, gels, and lotions. Akin to solid dosage products, dissolution is a key analytical technique used during semisolid product development to understand the effects drug substance (DS) and drug product (DP) properties have on product performance and as a quality and performance test.

The ever-increasing variety of drug product delivery systems that fall into the semisolid category continues to grow. This can lead to challenges when trying to understand the nature of the drug-release performance. Knowledge of the options available for semisolid product dissolution testing will help direct study experimental designs, whether for:

- lot release testing
- estimating product shelf-life in stability studies
- trying to correlate in-vitro test results to in-vivo drug action

SEMISOLID DOSAGE FORMS

- The challenge with semisolids is the wide variety of existing dosage forms, which includes gels, creams, pastes, lotions, suppositories, and transdermal patches (Figure 1). These products have a wide range of uses including therapeutic, protective, and cosmetic. Typically, semisolid products have one of two modes of application: topical or insertion into an orifice.[5,6] Products can be biphasic or monophasic and contain a wide range of components such as fatty acids, hydrocarbons, emulsifiers (anionic, cationic and nonionic), alcohols, synthetic polymers, lipid and more.[7] Semisolid products can also be hydrophilic or hydrophobic. These variables can make developing a dissolution method for semisolid dosage forms a daunting task.

Figure 1. Semisolid Dosage Forms[6]

SEMISOLID PRODUCT DISSOLUTION METHOD DEVELOPMENT

When developing a dissolution method, it is important to understand:

- The chemical composition of the drug product
- The mode of application
- The mechanism of transport
- The stage of research and development
- The purpose of the study and information desired, for example, drug release or transport data, or product performance for quality control release testing

Once these factors are explored, the method developer can choose to develop an IVPT or IVRT method:

- In Vitro Permeation Test (IVPT) is used in product development and focuses on mimicking biological conditions.
- In Vitro Release Test (IVRT) is used for stability and performance testing and is designed to assure consistent product quality.[8]

Whether developing an IVRT or IVPT for semisolid products, the method developer must choose a dissolution apparatus and a method of analysis for API quantification.

CHOICE OF DISSOLUTION APPARATUS

The choice of apparatus depends on: the purpose of the study, the drug product formulation, drug substance and its concentration, amount of sample required for testing, and the availability of equipment.

Analytical testing labs focused on supporting stability and product release testing commonly perform IVRT. Breaking the products into groups by application , i.e., topical or insertion (not discussed further), will help determine the dissolution approach and apparatus to use. For topical semisolid dosage forms like locations, creams, ointment, and pastes that are applied directly to the area to be affected or as an administering dose of API through the skin, the use of a system that provides a membrane barrier between the product and medium is required.

The three apparatuses that can accommodate this are:

- A vertical diffusion cell (VDC) apparatus, commonly called a Franz cell (Figure 2 and Figure 4)
- USP Apparatus 2 with an immersion cell apparatus, also called an enhancer cell (Figure 5)
- USP Apparatus 4 (flow-through cell) with an adapter sample holder (Figure 8).

These systems use a membrane as the barrier to separate the drug product and dissolution media. Each setup is described later in this paper.

When working with semisolid dosage forms, some terminology is a little different than with solid oral forms. The sample holders and media reservoirs are referred to as donor and receptor chambers, respectively. The dissolution media is referred to as the receptor medium and the material diffusing into the receptor medium is the permeant.

Often IVRT methods are developed and transferred to a Quality Control laboratory for product testing. When this is done, matching the type of instrumentation is essential. It is always a good practice to discuss approaches to analytical development with the lab that will be routinely running the method—the end-user.

As is customary with any dissolution method, IVRT and IVPT methods require the development of two methods that work together: a drug substance (DS) or API detection method and dissolution method.

API DETECTION METHOD DEVELOPMENT

The assay method must detect the API in the presence of the receptor media and any drug product matrix components. Fortunately, the matrix does not often interfere due to its dilution in the receptor medium. Development of a reliable analytical method for API assay is required prior to dissolution method development to evaluate dissolution parameters. The two most common approaches are Liquid Chromatography HPLC-UV and UV-Vis spectrometry. Others, such as radio labeling, are used as well. HPLC-UV analytical methods are often used when DP components interfere with the detection wavelength used to monitor API release. While fully automated dissolution and HPLC systems are available, automation is often simpler when UV-Vis spectroscopy can be used.

As with methods that support drug product or drug substance testing, these methods require full development and validation. The development parameters for early development or Phase I typically include specificity, linearity, accuracy, and precision (SLAP). For low concentration APIs, testing for a limit of quantitation (LOQ) is added. As the drug product moves to Phase II and Phase III, stability of standards and sample solution, intermediate precision, and robustness studies are also required.

The earlier these studies are performed, the better. For example, performing full robustness studies during development and understanding the limits of method parameters early in the process can eliminate unexpected method challenges later on. For extended release products with dissolution studies that last several days, knowing the stability of sample is essential. And, it is always a good idea to have a second analyst run a new method early in the development process.

DISSOLUTION METHOD DEVELOPMENT

Developing a robust method for IVRT and IVRP studies requires careful consideration of several parameters. These are: apparatus, receptor medium, membrane, medium temperature, agitation and rate, sample size and pull times.

Apparatus – The apparatus used can be determined from a few things, such as availability of equipment. The most commonly used system is the vertical diffusion cell or Franz cell. However, if your lab does not work with a lot of semisolid dosage forms and only has traditional dissolution equipment like USP Apparatus 2, then you will likely use this with an emersion cell (Figure 5). This option has the benefit of using existing equipment. A wide range of receptor chambers (vessels) from 100 mL to 1 L (for most systems) and the donor chambers (immersion cells) allow for different membrane surface area exposures and can accommodate various sample volumes in a controlled consistent manor. Apparatus 4 is typically used for very long dissolutions requiring flow through receptor medium.

Receptor Medium[9] – Choosing the receptor medium requires an understanding of the API and its solubility in the desired media. It is important to maintain sink conditions, often defined as having a saturation for the API in the receptor medium at least three times greater than the final concentration of the API in medium at the end of release.[10] Solubility studies are conducted to determine the effect of pH, salts, cosolvents, surfactants, and complexing agents on product solubility. The USP general chapter <1236> SOLUBILITY MEASUREMENTS provides background on the difference between solubility and dissolution rate, the solvent's capacity to dissolve a solute, and the rate to reach solubility limits. Methods to determine equilibrium solubility (such as the shake flask method, methods), and for determination of apparent solubility and solubility measurements in biorelevant media (such as Human Fasted-State Simulated Gastric Fluid (FaSSGF) or Human Fed-State Simulated Gastric Fluid (FeSSGF)) are also covered.

Membrane – The choice of membrane will be dependent on the type of study, IVRT or IVPT, which usually use synthetic and natural products, respectively. For IVRT methods, which are often QC methods, the membrane should be readily available from a reliable vendor. The material is often synthetic (such as nylon, silicone, cellulose, etc.) and should be inert with respect to the drug product components and APIs. It should not retain the active or be a barrier to release. The active released from the dosage form should move freely into the receptor medium and not be hindered by the membrane nor should the membrane contribute interferences to receptor medium impacting assay results.[11] Properties to consider are pore size, and hydrophobicity or hydrophilicity of the membrane.

Temperature – The temperature for the receiving chamber is normally 32 ± 1 °C or 37 ± 1 °C depending on whether the site of the administration is topical or internal, respectively.

Agitation – Agitation will be dependent on the apparatus. VDC uses stir bars with rpms typically at 600, and this is kept consistent across all diffusion cells. When appropriate, rpms ranging from 200 to 900 can provide proper mixing of the receptor fluid. The adequacy of stirring rates should be demonstrated during development and validation.[12,13] Enhancer or emersion cells use USP Apparatus 2 paddles to agitate the receptor medium. The rotation speed needs to be sufficient to maintain sink conditions at the membrane interface. For USP Apparatus 4, the flow rate would be set to accomplish this as well.

Sample Size and Pull Times – The sample size and pull times will depend on sample concentration and the study being performed. For example, it is common to sample a minimum of 5 time points over 24 hours when generating a release profile. The timing of each pull depends on the expected release rate. In general, early time points are spaced closer than later ones.

METHOD VALIDATION

The purpose of method validation is to demonstrate a method is suitable for its intended purpose. Validation guidelines and expected performance characteristics are available from FDA, ICH, and USP. The USP general chapter <1225> VALIDATION OF COMPENDIAL PROCEDURES, classifies methods into four categories and provides the data elements expected for validation in each category. As a performance test, IVRT methods are USP Category III. However, because IVRT methods include an assay method validated concurrently under one protocol, the validation elements include those from Category I and III (Table 1). Additionally, USP general chapter <1092> THE DISSOLUTION PROCEDURE: DEVELOPMENT AND VALIDATION provides detailed overviews of the elements required for the development and validation dissolution methods.

Table 1. Key Elements for Validation of a Dissolution Method

Element	Typical Approach
Specificity	Evaluation of sample matrix and assay system for interference and or evaluation of a material of similar composition that should be distinguished in the method.
Linearity	Assay series of standard to determine predictability of response mode
Accuracy	Spikes of sample matrix to determine recovery values usually at three levels
Precision	Repeatability – Assay multiple sample preparations to measure variability in use Intermediate – Precision prepared by a second analyst using separate equipment where possible Reproducibility - Precision prepared by a second analyst in a different lab
Range	Determined from acceptable precision and accuracy results
Robustness	Evaluate effect from small changes made to the method/system
Stability of Solutions	Demonstrate the stability of standards and samples

DISSOLUTION METHOD DEVELOPMENT

A well written method and a carefully thought out validation protocol is essential to a successful validation. The method, if only in draft form at this stage, should still be a controlled document to ensure consistent method operation throughout the validation process. The protocol that directs the validation studies must be approved by the sponsoring organization. Below is an excerpt from the FDA guidance document:

> **"The methodology and objective of the analytical procedures should be clearly defined and understood before initiating validation studies. This understanding is obtained from scientifically-based method development and optimization studies. Validation data must be generated under a protocol approved by the sponsor following current good manufacturing practices with the description of methodology of each validation characteristic and predetermined and justified acceptance criteria, using qualified instrumentation."** [14]

Two fundamental components of the validation are the use of qualified instrumentation and establishing predetermined acceptance criteria. These requirements make understanding the limits of both the method and instrumentation vital to a successful validation. This information is obtained by testing the method's limits during development and performing sufficient qualification studies prior to formal validation. If these steps are hurried due to time and budget constraints, there is a risk of additional work and delays during validation due to unexpected results. If the validation reveals the need for method changes, these can be documented and the method updated upon successful completion of the validation.

SEMISOLID PRODUCT DISSOLUTION APPARATUSES

Dissolution Apparatuses Used for Semisolid Dosage Forms

There are three USP apparatuses that are commonly used for the dissolution of semisolid dosage forms:

- A vertical diffusion cell (VDC), commonly referred to a Franz cells
- USP Apparatus 2 with an immersion cell apparatus, also called and enhancer cell
- USP Apparatus 4 (flow-through cell) with an adapter sample holder

Vertical Diffusion Cells (VDC)

The VDC or Franz diffusion cell is commonly used for in-vitro studies and was described by Dr. Thomas J Franz back in 1978.[15] This apparatus has a donor chamber situated directly above the receiver chamber with a membrane separating the two (Figure 2). Side-by-side systems (Figure 3) are also available.[16]

The drug product is applied directly to the membrane in the donor chamber and the drug substance passes through the membrane into the receptor fluid in a temperature controlled receiving chamber. Typically, the temperature is held at 32 ± 1 °C or 37 ± 1 °C depending on the mode of the administration topical or internal, respectively. Membrane materials can consist of excised human or animal skin, synthetic material, or a synthetics and skin combination. Often membranes are presoaked prior to use for 30 minutes.

To prevent the product from drying out, the donor chamber can be sealed with a non-permeable film. The donor and receiving chamber are available in varying sizes from 5 to 20 mL.[17] The receptor media volume is maintained through the sampling port that allows for the withdrawal of sample for analysis and media replacement. Receptor fluid is keep in contact with the membrane and stirred with a stir bar or helix mixer (Figure 4) to prevent a concentration build up near the membrane and maintain a steady state of diffusion across the membrane surface.

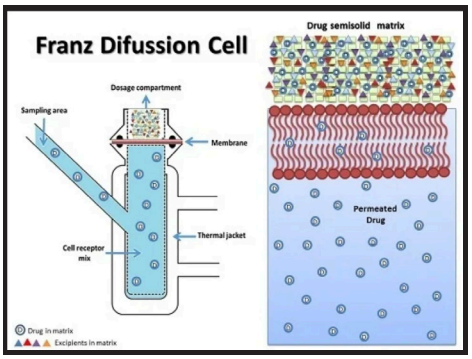

Figure 2. Franz Diffusion Cell[18]

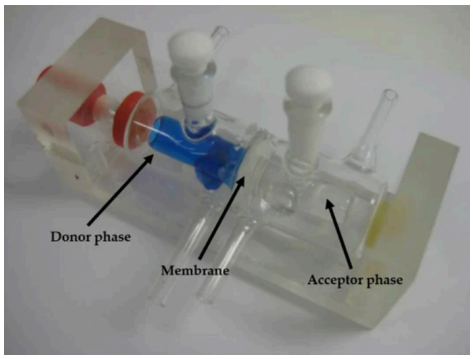

Figure 3. Side-By-Side Cell[16]

Figure 4. Franz Cells with Helix Mixers[19]

The challenge with these cells is making sure the sample and receptor fluid maintain contact with the membrane. Being mindful of air bubbles is critical. Samples pull times can vary depending on the type of study. For a profile determination, early pulls may be every half hour, then hourly and daily with the level of the media being check prior to sampling and replaced after sampling.

USP <1724> describes three VDC models A, B, and C (not shown). They all have the same general design as a Franz cell with different dimensions and sealing mechanisms for the receiving chambers. A notable difference is the helix shaped stirring mechanism for Model A, whereas Model B and C use a simple stir bar.

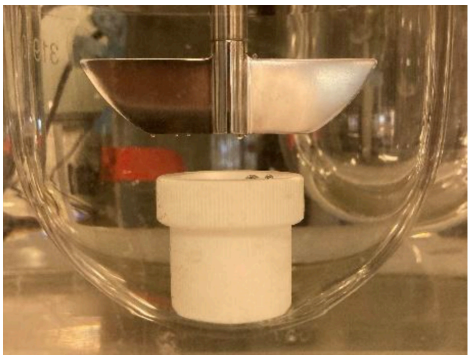

Figure 5. Agilent Enhancer Cells in USP Apparatus 2 Dissolution Vessel

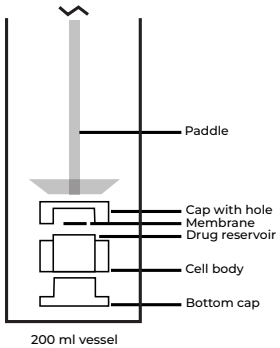

Figure 6. Schematic of an Immersion Cell in Reduced Volume Vessel

USP APPARATUS 2 WITH IMMERSION OR ENHANCER CELL APPARATUS

Immersion or enhancer cells provide a controlled mechanism to perform in vitro release testing using a traditional Apparatus 2 dissolution system. This is accomplished by placing an immersion cell filled with the drug product directly under the pad in an Apparatus 2 vessel (Figure 5). The Apparatus 2 is then run as in traditional dissolution with the paddles rotating to keep the receptor media moving. Sample pulls and analysis can be manual or automated depending on the system setup.

While immersion cells may have slightly different designs, they all have a donor chamber where one side is a membrane and the remaining sides are sealed to prevent media from contacting the DP (Figure 6). The vessel system is designed to ensure a consistent fill between cells. The top of the cell has a hole or window that exposes the membrane to the receptor media and holds the membrane in place when screwed onto the cell body. A retaining ring makes a leak resistant seal. The bottom cap can be place into the cell body to a fixed position, causing the drug product to make solid contact with the membrane and squeeze out any air bubbles. All the components are easy to separated and clean.

The immersion cell can be used with a range of receptor chambers (vessels). For Apparatus 2, the typical standard size is 100 mL to 1 liter, with larger systems available. The 200-mL chamber is a common and convenient size. The use of a flat bottom or dimpled vessel prevents the dead space that would occur at the bottom of a round bottom vessel.

USP APPARATUS 4

Apparatus 4 is a flow-through cell method designed in 1957 by the FDA and adopted by the USP for the evaluation of controlled release products. Apparatus 4 dissolution runs can last for several days, weeks, and even months. Apparatus 4 has a temperature controlled reservoir for the receptor medium, which is pumped up through the vertically positioned flow-cell containing the drug product (Figure 7).

These systems can be open loop or closed loop (Figure 8). A closed loop system circulates the same medium through the cell whereas the open loop system consistently passes fresh receptor medium through the cell and collects the medium for analysis.

When using Apparatus 4 to test semisolids, an adapter cell filled with DP is placed in the flow cell. The adapter provides a means to consistently expose equivalent amounts of DP to receptor media through a membrane. The adaptor cell volume are typically a few hundred microliters to just over a milliliter.[20]

Figure 7. Apparatus 4 Flow-Through Cell

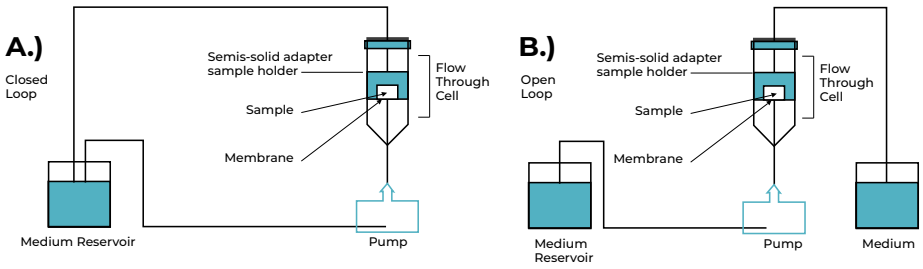

Figure 8. Schematic of Apparatus 4 with Semi-Solid Adapter Sample Holder A) Closed Loop, B) Open Loop

SUMMARY

Although the USP general chapter <1724> has only been in effect for a decade, in-vitro testing of semisolids has been an established technique for over 60 years.[21] The wide variety of drug product delivery systems and formulations that fall into this category continues to grow.

An understanding of the instrumentation available for the development of IVPT and IVRT methods helps overcome the challenges in developing and validating robust methods that reveal the nature of the drug-release performance. Through the use of enhancer and adapter cells, IVRT methods can be developed using traditional USP dissolution systems like Apparatus 2 and Apparatus 4.

In addition, familiarity with the options available for dissolution testing will help direct study experimental designs, whether for:

- lot release testing
- estimating product shelf-life in stability studies
- correlating in-vitro test results to in-vivo drug action

Semisolids continue to be developed as a favorable drug delivery mechanism. As semisolids are developed, the techniques used to test those products will also develop further, and dissolution will be in demand as one of the best means to ensure product safety and efficacy.

REFERENCES

1 Sotax, Dissolution Testing, accessed 15Jun22, https://www.sotax.com/dissolution_testing.

2 What is the USP dissolution test?, accessed 09Jun22, https://www.usp.org/chemical-medicines/dissolution explained#:~:text=In%20its%20more%20than%2040,to%20the%20variability%20of%20results.

3 Alley, S.; The role of dissolution in drug development, Outsourcing-Pharma.com, 25Sep16 Last updated on 29Sep16 at 12:49 GMT.

4 Spisak, D.; Dissolution Testing of Gels, Topical Creams & Ointments, Agilent Technologies, Practical Solutions Newsletter, Volume 15 Issue 4.

5 Toro, J.L., Semi-Solids: A Regulatory Perspective, accessed 25Aug22, https://www.contractpharma.com/issues/2018-06-01/view_fda-watch/semi-solid-dosage-forms/.

6 Adepu, S.; Ramakrishna, S. Controlled Drug Delivery Systems: Current Status and Future Directions. Molecules 2021, 26, 5905. https://doi.org/10.3390/molecules26195905.

7 Shrestha, H.; Bala, R.; Arora, S.; "Lipid-Based Drug Delivery Systems", Journal of Pharmaceutics, vol. 2014, Article ID 801820, 10 pages, 2014. https://doi.org/10.1155/2014/801820.

8 Heaney, J.; Diffusion Method Development Guidelines, Hanson, 0001 Rev A, 22 May (2014) 1-13.

9 Su, P.H.; Wang, K.; IVRT Insights From CMC Experts Offrontage Labs, February 20, 2020 accessed August 25, 2022, https://www.frontagelab.com/wp-content/uploads/2020/03/White-Paper_CMC-IVRT.pdf.

10 Phillips, D.J.; Pygall, S.R.; Cooper, V.B.; Mann, J.C.; Overcoming sink limitations in dissolution testing: a review of traditional methods and the potential utility of biphasic systems. Royal Pharmaceutical Society 2012 Journal of Pharmacy and Pharmacology, 64, pp. 1549–1559.

11 Dereli, H.G.; Şengün, E.K.; Genç, C.O.; Arslan, B.Ö.; Gökalp, M.U. Method Development and Validation of in Vitro Release Test for Fluorometholone Ophthalmic Suspension using Franz Diffusion Cell System. Glob J Pharmaceu Sci 9(2): GJPPS.MS.ID.555758 (2021).

12 Rath, S; Kanfer, I. A; Validated IVRT Method to Assess Topical Creams Containing Metronidazole Using a Novel Approach. Pharmaceutics. 2020 Feb 3;12(2):119. DOI:10.3390/pharmaceutics12020119. PMID: 32028557; PMCID: PMC7076423.

13 Klein, R.R.; Heckart, J. L.; Thakker, M.U.; In Vitro Release Testing Methodology and Variability with the Vertical Diffusion Cell (VDC).Dissolution Technologies, August 2018 www.dissolutiontech.com dx.doi.org/10.14227/DT250318P52.

14 U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Analytical Procedures and Methods Validation for Drugs and Biologics, Guidance for Industry, July 2015 pg 7.

15 Franz, T.J.; The finite dose technique as a valid in vitro model for the study of percutaneous absorption, Curr. Probl. Dermatol., 7 (1978) 58-68.

16 Bartos, C.; Szabó-Révész, P.; Horváth, T.; Varga, P.; Ambrus, R.; Comparison of Modern In Vitro Permeability Methods with the Aim of Investigation Nasal Dosage Forms. Pharmaceutics 2021, 13, 846. https://doi.org/10.3390/pharmaceutics13060846.

17 PermeGear, Franz Cell – The Original, accessed 10Aug22, https://permegear.com/franz-cells/.

18 Salamanca, C.H.; Barrera-Ocampo, A.; Lasso, J.C.; Camacho, N.; Yarce, C.J.; Franz Diffusion Cell Approach for Pre-Formulation Characterisation of Ketoprofen Semi-Solid Dosage Forms. Pharmaceutics 2018, 10, 148. https://doi.org/10.3390/pharmaceutics10030148.

19 Bajza, Á.; Kocsis, D.; Berezvai, O.; Laki, A.J.; Lukács, B.; Imre, T.; Iván, K.; Szabó, P.; Erdő, F. Verification of P-Glycoprotein Function at the Dermal Barrier in Diffusion Cells and Dynamic “Skin-On-A-Chip,” Microfluidic Device. Pharmaceutic, 2020, 12, 804.https://doi.org/10.3390/pharmaceutics12090804.

20 Kailas Thakker, Ryan Klein, Chapter 19 - Drug release: topical products, Editor(s): Christopher M. Riley, Thomas W. Rosanske, George Reid, Specification of Drug Substances and Products (Second Edition), Elsevier, 2020, Pages 505-534, ISBN 9780081028247, https://doi.org/10.1016/B978-0-08-102824-7.00019-1.

21 Markovich, R.J.; Dissolution Testing of Semisolid Dosage Forms, American Pharmaceutical Review, (2001) 71-79.