Review Article

Theme: Critical Variables in the In Vitro and In Vivo Performance of Parenteral Sustained Release Products

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Points to Consider when Establishing Drug Product Specifications for Parenteral Microspheres

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Abstract. Drug product specifications are a critical element of a good control strategy. Parenteral microsphere products are complex dosage forms, requiring careful development of test methods and acceptance criteria for the specifications. In particular, the *in vitro* release test method and acceptance criteria require rigorous scientific consideration and should be developed with an eye toward understanding the mechanisms of drug release. The final specifications need to ensure the safety, identity, strength, performance, and quality of the drug product at release and during storage through the end of its shelf-life. The specification limits are typically established based upon regulatory guidance, available data from the manufacturing process (process capability), from non-clinical, clinical, and stability studies.

KEY WORDS: control strategy; *in vitro* mechanism of release; microsphere drug product attribute; microsphere specification; parenteral microspheres.

INTRODUCTION

Drug-loaded parenteral microspheres are a growing class of formulations that impart prolonged residence of therapeutics in the body with significant therapeutic, adherence, and convenience benefits to patients. The development and manufacture of these types of products is complex and requires significant formulation, process, and analytical development efforts for success. Product specifications are central to a successful control strategy. This article summarizes the critical attributes that are relevant to establishing drug product specifications as part of a control strategy for injectable microspheres. Specific focus will be placed on testing and acceptance criteria development for the *in vitro* release profile. Important points to consider when establishing test methods and acceptance criteria are also discussed.

The concept of using biodegradable polymers for sustained release parenteral drug delivery began in early 1970s (1). The application of biodegradable microspheres to deliver small molecules, proteins, and macromolecules using multiple routes of administration has been widely investigated and several products have been brought to market in the last 10–20 years. A list of marketed injectable products is shown in Table I.

A reasonably wide range of microencapsulation techniques have been developed to date. The selection of a particular technique is dependent on the physicochemical properties of the drug, polymer, and the intended use of the drug product. Several examples of commercially successful techniques include: (a) phase separation, (b) double emulsion, (c) spray-drying, and (d) cryogenic spray-drying (Pro-Release® Technology). The most widely used technique, the conventional emulsification process, is illustrated in Fig. 1. The microsphere product in general, is supplied as a dry powder that is re-suspended in the supplied vehicle (vial, pre-filled syringe, or a dual chamber device) prior to injection.

Microspheres are typically manufactured by an aseptic process because terminal sterilization (heat sterilization and gamma irradiation) results in degradation of the microspheres, loss of polymer molecular weight, and hence, performance. Due to the complexity in the development and manufacturing process for microsphere drug products an appropriate quality control strategy should be designed during the development program. This strategy should include thorough product characterization during development, adherence to good manufacturing practices; e.g., suitable facilities, a validated manufacturing process, validated test procedures, raw material testing, in-process testing, stability testing, and other use testing, and of course drug product specifications. Specifications are designed to ensure product quality and consistency, and are typically established based upon regulatory guidance, available data from the manufacturing process (process capability), from non-clinical, clinical, and stability studies.

According to ICH guidelines (5), a specification is defined as a list of tests, references to analytical procedures,

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Table I. List of Marketed Microsphere Drug Products (2–4)

Drug	Commercial name	Company	Technology	Indication
Risperidone	RISPERDAL® CONSTA®	Janssen®/Alkermes, Inc.	Double emulsion (oil in water)	Schizophrenia; bipolar I disorder
Naltrexone	Vivitrol®	Alkermes	Double emulsion (oil in water)	Alcohol dependence
Leuprolide	Lupron Depot® Enantone Depot® Trenantone® Enantone Gyn	TAP Takeda Takeda Takeda	Double emulsion (water in oil in water)	Prostate cancer/endometriosis
Octreotide	Sandostatin® LAR	Novartis	Phase separation	Acromegaly
Somatropin	Nutropin® Depot ^a	Genentech/Alkermes	Alkermes ProLease® Technology (Cryogenic spray-drying)	Growth deficiencies
Triptorelin	Trelstar™ depot Decapeptyl® SR	Pfizer Ferring	Phase separation	Prostate cancer
Buserelin	Suprecur® MP	Sanofi-Aventis	N/A	Endometriosis
Lanreotide	Somatuline® LA	Ipsen-Beafour	Phase separation	Acromegaly
Bromocriptine	Parlodel LAR™	Novartis	Spray dry	Parkinsonism
Minocycline	Arestin®	Orapharma	N/A	Periodontitis

N/A information not available

^a Withdrawn from market voluntarily by the manufacturer

and appropriate acceptance criteria, which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug product should conform to be considered acceptable for its intended use. Furthermore, specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval.

Fig. 1. Flow diagram for manufacturing microspheres by conventional emulsification process

The two fundamental aspects of setting specifications are definition of the critical attributes and establishment of criteria for accurately controlling each attribute (6). The drug product specifications are selected to ensure product quality rather than to establish full characterization, and should focus on those characteristics found to be essential to ensure safety and efficacy.

In this article the authors are expanding on the guidance given in ICH/FDA documents by adding specific points to consider for the various attributes and test methods as it relates to drug product specifications for parenteral microspheres. Unlike controlled release oral formulations, there are no generally preferred acceptance criteria for the *in vitro* release profile for parenteral microspheres drug products, likely due to the fairly wide variety of intended product profiles in terms of injection site and *in vivo* release profile. For this reason, specific focus is placed on testing and acceptance criteria development for the *in vitro* release profile.

DRUG PRODUCT ATTRIBUTES

The selection of drug product attributes and the corresponding test methods should be adequate to ensure quality and consistency of the drug product, which includes safety, efficacy, identity, strength, performance (i.e., initial and complete release profiles), and quality of the drug product at release and during storage through the end of its shelf-life.

The drug product attributes tested at release for a typical microsphere drug product are listed in Table II along with examples of test methods, acceptance criteria, primary function, and if the attribute is typically stability indicating. The final specification limits for the commercial product should be based on release and stability data generated throughout development, manufacturing process (process capability), analytical capability, non-clinical and clinical exposure, and regulatory guidelines. It is important that stability be monitored during development, and, for the final drug product, appropriate acceptance criteria are established for the stability indicating attributes.

Table II. Microsphere Drug Product Attributes

Attribute	Example method	Example acceptance criteria	Function	Stability indicating
Aspect appearance	Visual	<i>Product</i> : white to off-white powder <i>Suspension of product</i> : suspends in diluent without clumping <i>Injection of the product</i> : product passes through the needle smoothly with little to no resistance	Quality	Yes
Identification	HPLC and FTIR or mass spectrometry	Retention times/spectrum of a sample relative to a standard mass is $\pm X$ Daltons	Identity	No
Assay	Chromatography or spectroscopy	Acceptance criteria and ranges could be developed in terms of milligrams of drug per 100 mg of microspheres	Strength	Yes
Purity	HPLC	Total and individual related substances	Quality	Yes
<i>In vitro</i> release	Static, methods using dialysis membrane; samples taken and measured by HPLC or UV spectroscopy	Initial release: NMT $X\%$ Complete release (real time or accelerated): 3–5 time-points depending on the release mechanism	Quality/product performance	Yes
Polymer molecular weight	Gel permeation chromatography	Range X – Y kD	Quality/product performance	Yes
Particle size	Laser diffraction	$Dv_{10} > w$, $Dv_{50} x-y$, and $Dv_{90} < z$	Quality/product performance	No
Water content	Volumetric/coulometric Karl Fischer, near-IR	NMT $X\%$	Quality	Yes
Content uniformity	HPLC or UV spectroscopy	USP <905>	Strength	No
Bacterial endotoxins	LAL	NMT XX EU/mg	Safety	No ^a
Internal/external sterility	USP <71>	No evidence of microbial growth	Safety	No ^a
Residual solvents	Gas chromatography	Follow ICH guidelines and process capability	Safety	No

LT less than, *NMT* not more than, Dv_{90} 90% particles by volume smaller than, Dv_{50} 50% particles by volume smaller than, Dv_{10} 10% particles by volume smaller than

^a Bacterial endotoxins and sterility are not stability indicating but are required on stability because it is a sterile product; container closure integrity (CCI) test may be employed in lieu of sterility test during stability (7)

Aspect and Appearance

Aspect is a qualitative test that could be developed to control product quality by including visual inspection of the integrity of the vial package, the appearance of the microspheres, and the testing of the product with the delivery system. For example, microsphere suspension and injectability; product performance characteristics can also be explored under aspect and these elements could be quantified for syringability (ease of aspiration into syringe) and injectability (ease of injection) by appropriate techniques (8).

Identification

Drug product identification can be performed using two different methods such as HPLC and FTIR or a single method such as mass spectrometry. Acceptance criteria may be the comparison of retention time of the main peak of a

reference standard to that of the main peak in the drug product sample and spectrum of the reference standard to that of the drug product sample or determining the mass of the encapsulated drug substance.

Assay/Purity

Assay is a quantitative procedure that controls product strength (% label claim; coreload). It is reported as the amount of total drug in milligrams encapsulated per 100 mg of microspheres. It may also be referred to as encapsulation efficiency, which is the actual amount encapsulated with reference to the theoretical load. The method should be capable of discriminating the amount of encapsulated drug from un-encapsulated portion. Appropriate extraction method should be developed to ensure acceptable recovery of both active and any potential impurities and/or degradation products. Although a single method to quantify assay

Fig. 2. Representative *in vitro* release and microsphere molecular weight profile at 37°C

and purity is desirable, in many cases multiple chromatographic techniques may be needed to control levels of key impurities.

In Vitro Release Methods

The *in vitro* release is a key quality attribute to evaluate and demonstrate acceptable product performance. Unlike the situation for oral formulations, there are no standardized test procedures (e.g. USP methods) for parenteral microsphere products. A proper *in vitro* test typically involves a more complex methodology and the process of establishing acceptance criteria is usually more complicated. In addition, the *in vitro* release methods should be designed to be biorelevant in that they mimic the duration and release patterns seen *in vivo* in appropriate animal models. The authors provide a specific example below to illustrate the types of points to consider when developing the specifications.

This test may involve one or more methods or a combination of methods. Although there are a number of different methods reported in the literature to evaluate the *in vitro* release profile (3), it is essential to identify a method that is most relevant to the mechanism of release and quantifies the different phases of the release profile. The primary goal is to use the *in vitro* test for pharmaceutical process and product control. Since microsphere long-acting dosage forms are typically designed to release over periods of weeks, months or even years, it becomes impractical to wait for a real-time test for batch release of product. Therefore, accelerated methods are often developed to assist in evaluation and batch release of product. The accelerated methods must increase the rate of release without affecting the drug release mechanism.

In order to set appropriate specifications for the *in vitro* release, the mechanism of release needs to be fully characterized. In addition, the discriminatory capability of the methods and the relevance of the *in vitro* release methods to *in vivo* product performance should be determined. A typical release mechanism for these types of products includes three phases of release and the release mechanism for both real-time and accelerated methods (higher temperature) is generally comparable. The three phases of release can be generally represented as the initial release phase (phase 1), the hydration phase (phase 2), and primary release phase (phase 3) that is diffusion controlled but facilitated by erosion

of the polymer matrix. Figures 2 and 3 illustrate representative *in vitro* release curves at 37°C and 45°C, respectively, with the three release phases identified and the corresponding observed polymer molecular weight values indicated. Quantitation of the cumulative release profile at multiple time-points provides a means of assessing the drug product performance. For example, several time-points with the appropriate criteria should be considered to describe the entire profile. An early time-point in the first few days should be considered for controlling initial release. The specific criteria are often driven by clinical safety. A time-point within the early release phase of the profile (phase 2) should be considered which would correspond to days 15–21 in Fig. 2 and days 5–10 in Fig. 3. A “not more than” specification could be considered for this time-point. Suitable time-point(s) on phase 3 of the profile during days 28–31 in Fig. 2 and days 10–12 in Fig. 3 could be selected to ensure consistency during the primary release period. The final time-point should be selected so as to ensure that the drug is fully released from the dosage form. The criteria for full release are generally set at a minimum of 80% of total drug.

Mechanism of Release

The mechanism of release in general, is studied by sampling at various time-points along the release profile of the two complete *in vitro* release test conditions (temperatures of 37°C and 45°C, in the above example) and analyzed for polymer molecular weight. This is augmented by evaluation using scanning electron microscopy and light microscopy. The authors suggest that sponsors provide information on the understanding of the release mechanism to support and justify accelerated stability tests.

Phase 1 Release—initial release

When the microspheres come into contact with an aqueous medium, the microsphere surface becomes wetted and any loosely bound surface drug is readily dissolved and released into the medium. This constitutes the initial release phase and is characterized by a slight “burst” of drug into the medium.

Phase 2 Release—hydration phase

Over a period of time following initial release, very little drug is released. During this phase the microspheres continue

Fig. 3. Representative *in vitro* release and microsphere molecular weight profile at 45°C

Fig. 4. Scanning electron microscopy images showing cross-sections of microspheres incubated in *in vitro* release medium at 37°C

to hydrate and undergo a steady decrease in polymer molecular weight. Incubation temperature has a strong influence on the rate of molecular weight decrease, in that higher temperatures shorten this phase from approximately 21 days at 37°C (Fig. 2) to approximately 7 days at 45°C (Fig. 3). When examined by scanning electron microscopy throughout phase 2, cross-sectional images of the microspheres show increasing internal pore diameter and progressive erosion of the polymer matrix (Fig. 4).

Phase 3 Release–Primary release phase

In the above example, after approximately 21 days at 37°C (or 7 days at 45°C), polymer molecular weight decreases below a threshold level of approximately 5–10 kD. At this point the encapsulated drug continues to be released in a controlled fashion until complete release is reached. The release is likely facilitated by a combination of drug diffusion through the highly degraded matrix and direct drug exposure to the medium as a result of microsphere erosion.

Discriminatory Capability of the *In vitro* Release Method

The release characteristics of an extended release microsphere formulation are a function of the physicochemical properties of the drug substance (e.g., aqueous solubility, solid-state characteristics, interactions with the polymer, etc.) as well as the parameters defining the microsphere formulation (e.g., polymer characteristics, drug load, etc.). The key formulation parameters controlling drug release are drug load (ratio of drug to polymer), PLG monomer ratio (polymer type), and PLG polymer molecular weight. Different release profiles can be achieved through changing manufacturing process conditions and by varying the attributes of the polymer used for manufacturing. The *in vitro* release method should be able to discriminate formulation and process changes that could affect product performance. An example of such is shown below in Fig. 5. In this study the effect of polymer type and drug load were evaluated in different formulations (A to F) using an accelerated *in vitro* test method. The results showed that the *in vitro* release method is able to discriminate between formulation differences during

all phases of *in vitro* release that could result in changes to the product performance. To reiterate the point, the objective of a discriminating *in vitro* release test is assessment of process control and reliability of performance of the product from batch to batch.

During development, it is essential to generate *in vitro* release profiles and understand the phases of the release mechanism. Appropriate time-points must be identified to support specifications for batch release and need to be based on the understanding of the mechanism of release, encompassing the different phases of the release profile.

Polymer Molecular Weight

The molecular weight of the drug product is a key attribute as it affects product performance (e.g. initial release and duration of release). The molecular weight may be affected during the manufacturing process and on stability; for example, high shear processing such as homogenization or ultrasonic mixing of polyester microspheres would lead to significant decrease in polymer molecular weight during microsphere processing (9). Also, the encapsulated material (e.g. meperidine) may catalyze the hydrolytic cleavage of the polymer during the manufacturing process and on stability

Fig. 5. *In vitro* release profiles for formulations differing in polymer matrix and drug load

causing the polymer to degrade (i.e. decrease in molecular weight), which usually results in a faster release of drug (10).

The polymer molecular weight determination is a quantitative method that may involve determining the weight-average molecular weight relative to appropriate standards (e.g. polystyrene) using gel permeation chromatography. The establishment of the polymer molecular weight specifications for the drug product should ensure acceptable product performance (i.e. *in vitro* release) at release and throughout its shelf-life.

Particle Size

Particle size can be measured by a variety of techniques and most commonly by laser diffraction using a light scattering instrument. The control of particle size is important to ensure consistency of batches. Additionally the effect of particle size on *in vitro* release needs to be determined in order to establish appropriate specifications.

Water Content

The water content is a key test as polymeric microspheres in general are degraded by hydrolysis. Several typical methods may involve Karl Fischer titration or near-IR spectroscopy at release and during stability (shelf-life) to ensure the quality of the drug product and to demonstrate that no significant change in water content occurs during storage.

Content Uniformity

In order to test uniformity of dosage units of the drug product batch at release appropriate methods should be developed. These methods do not need to be selective or stability indicating. A sampling plan and acceptance criteria per USP <905> (11) should be considered and established.

Residual Solvents

Residual solvents analysis is essential if the manufacturing process involves organic solvents to ensure that the drug product has acceptable solvent levels. The limits should be established for specific solvents giving consideration to patient safety and product performance (ICH guidelines and process capability). Methods using gas chromatography and near-IR are typically used.

Bacterial Endotoxins

The bacterial endotoxins specification limits should be established to ensure safety. Bacterial endotoxin testing is performed per USP <85> using the limulus amoebocyte lysate and calculation for endotoxin limits may be based on the USP guidance.

Sterility

Microspheres in general are manufactured by an aseptic process because several other widely used techniques (heat sterilization and gamma irradiation) result in degradation of

the microspheres, loss of molecular weight. In order to assure sterility, sterility testing is performed per USP <71> using direct inoculation. Two types of sterility tests are performed (a) external—to test for the absence of microorganisms within the finished drug product vial and (b) internal—to ensure the absence of microorganisms encapsulated in the microspheres, and usually performed by dissolving the microspheres in a solvent that does not exhibit anti-microbial properties (12).

SUMMARY

Drug product specifications are a critical element of a good control strategy. Parenteral microsphere products are complex dosage forms, requiring careful development of test methods and acceptance criteria for the specifications. In particular, the *in vitro* release test method and acceptance criteria require rigorous scientific consideration and should be developed with an eye toward understanding the mechanisms of drug release. The final specifications need to ensure the safety, identity, strength, performance, and quality of the drug product at release and during storage through the end of its shelf-life. The specification limits are typically established based upon regulatory guidance, available data from the manufacturing process (process capability), from non-clinical, clinical, and stability studies.

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