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Performance Test for Parenteral Dosage Forms

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ABSTRACT

Procedures and acceptance criteria for testing parenteral drug products are divided into two categories: (1) those that assess product quality attributes (e.g., identification, sterility, and particulate matter) and are contained in general chapter Injections and Implanted Drug Products <1>; and (2) those that assess product performance (e.g., in vitro release of the drug substance from the drug product). A product performance test (i.e., drug release test) should be carried out using appropriate test parameters and procedures. This Stimuli article addresses performance tests for parenteral drug products and the various methodologies.

INTRODUCTION

Parenteral drug products include solutions, suspensions, emulsions, sterile powders for solutions or suspensions (including liposomes), implants (including microparticles), and products that consist of both a drug and a device, such as drug-eluting stents. Two categories of tests, product quality and product performance, are performed on such drug products. These tests provide assurances of batch-to-batch quality, reproducibility, reliability, and performance. Product quality tests are performed to assess attributes, such as assay, identification, and content uniformity, and are part of the compendial requirements (see general chapter Injections and Implanted Drug Products <1>). Product performance tests are conducted to assess drug release from the dosage form.

Parenteral drug products such as solutions, suspensions, and emulsions are relatively simple in comparison to microsphere and liposome preparations. For the less complex products, a simple drug-release test performed with Apparatus 2 (paddle method) can be used. However, the use of Apparatus 4 (the flow-through cell) may be a preferred method for coarse suspensions or emulsions. Dialysis sac and reversed dialysis sac methods can also be used for emulsions.

Parenteral products, such as microspheres and liposomes, are complex in nature, which complicates the development and validation of assays for in vitro drug release.

Methodologies can be developed, validated, and standardized, but because of the complexity of the various

types of parenteral drug products, a single method for determining drug release is not currently feasible, although a significant amount of research is being conducted in this regard. An American Association of Pharmaceutical Scientists workshop report recommended the use of Apparatus 4 for evaluating the release profile of novel systems, such as nanoparticles and liposomes (1).

In the development of dissolution/drug-release tests or the selection of the drug-release medium, apparatus, procedure, or analytical method, consult general information chapter The Dissolution Procedure: Development and Validation <1092>. In developing the performance test specifications, one should consider the mechanism of drug release as well as the drug's mechanism of action (2). In vitro release (IVR) is a key performance attribute used to evaluate and demonstrate product performance; IVR is used for process and product control. Long-acting drug products are typically designed to release the drug over an extended period of time. Accelerated methods are necessary to assist in the evaluation of these drug products without affecting the drug-release mechanism (3, 4).

PERFORMANCE TEST METHODS FOR PARENTERAL DOSAGE FORMS

For most parenteral drug products, the performance test includes drug release from the formulation. The performance test may include the dialysis sac, reverse dialysis sac, manual shaker bath (sample and separate), paddle method (Apparatus 2), flow-through system (Apparatus 4), or other methods for testing in vitro release. Standardized methods such as Apparatus 2

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and 4 are preferred to allow data comparison between laboratories.

In Vitro Drug-Release Testing Using the Dialysis Sac Method

The dialysis sac method involves placing the formulation (e.g., liposomes) into a dialysis sac or tube. This dialysis sac is then placed in a large volume of release medium (receiver chamber). The receiver chamber is stirred to ensure mixing of the released drug. However, the inside of the dialysis sac is not stirred, and this can result in aggregation problems as well as data that cannot be reproduced. The drug released from the formulation diffuses through the dialysis sac membrane, and the drug concentration is determined by taking samples from the release medium at appropriate time intervals. The temperature of the entire chamber is maintained at 37 ± 2 °C using a water bath (5).

Selection of Membrane

Cellulose ester, regenerated cellulose, and polyvinylidene difluoride are the most commonly used membranes for the dialysis sac. The membrane should have appropriate molecular weight so that drug diffusion through the membrane is not a rate-limiting step.

Conditioning of Membrane

The dialysis membrane should be conditioned through soaking in the release medium for an appropriate length of time. This helps remove any extractable substance from the membrane that could potentially interfere with the analysis.

Sample Introduction

The formulation can be introduced to the dialysis sac using a syringe or fine-tip pipet. The formulation can be placed in the dialysis sac directly or can be mixed with a small volume of release medium.

Agitation

Agitation can be achieved using a shaker water bath or a magnetic stirrer. Appropriate control of hydrodynamics is important for reproducible results.

Temperature

The temperature of the entire chamber is usually maintained at 37 °C throughout the test. The temperature should be calibrated using a certified thermometer and held at 37 ± 0.5 °C.

Volume for Drug-Release Testing

When selecting the volume of the medium outside of the dialysis sac, the analyst should take into account the solubility of the active pharmaceutical ingredient

(API) and sink conditions. Replenishing the medium at various stages of the test may be necessary to achieve sink conditions. When drug release is rapid, it may be impossible to maintain sink conditions within the dialysis sac, and as a result, the dialysis sac becomes a rate-limiting membrane (6, 7).

Sampling Parameters

Sampling time points should be chosen to adequately describe any initial burst release and provide an adequate number of data points to create a drug release profile. Sampling should occur within ± 2 min for time points up to 12 h; within ± 5 min for time points between 12 and 24 h; and within ± 30 min for later time points. It is important that the medium is mixed adequately during sampling so that a representative sample is obtained. The medium may be replenished after sampling.

Medium for Drug-Release Testing

The medium should be chosen with consideration of the physical and chemical characteristics of the drug substance and dosage unit. See Chapter <1092> for further guidance. The medium should be degassed using an appropriate technique unless the use of surfactants is necessary. A specified volume of medium should be transferred accurately to the receiver chamber. Media should be stirred throughout the test.

Analysis and Calculations

The assay for a dissolution sample is HPLC, although other methods, such as spectrophotometry, may be used if feasible. On-line fiber-optic UV probes are useful for fast-releasing formulations because complete characterization of the release profile is possible (more data points can be collected in short time intervals).

A cumulative release profile can be obtained by analyzing samples at appropriate time points; the concentration ($\mu\text{g/mL}$ or mg/mL) of the analyte is determined at each time point using appropriate analytical methods. The concentration is then multiplied by the total volume of the dissolution medium, thereby determining the total amount released during different time intervals. This allows determination of the cumulative amount released. Calculations should account for all dissolution medium adjustment and replenishments.

In Vitro Drug Release Using the Reverse Dialysis Sac Method

The reverse dialysis sac method involves placing the formulation directly into an appropriate volume of release

medium in a suitable chamber. Two or more dialysis sacs are placed in the release medium. At appropriate time intervals, one dialysis sac is removed, and the drug concentration is analyzed to calculate the percentage of release. This approach helps to maintain sink conditions because the formulation is diluted in the large volume of release medium outside the dialysis sacs (5, 6, 8). In addition, there are no problems of aggregation of the delivery system due to lack of direct stirring within the dialysis sacs (as can occur in standard [i.e., not reverse] dialysis methods).

In Vitro Drug Release Using Apparatus 2

The use of Apparatus 2 may be an effective way to perform in vitro drug-release testing for some parenteral formulations. Depending on the hydrophobic/hydrophilic nature of the drug product and its molecular weight, floating and/or aggregation of the formulation may occur. When aggregation occurs, it is justified to use another apparatus to perform the in vitro release testing. Modifications to the medium during a run are more challenging with Apparatus 2 than with Apparatus 4, and for this reason Apparatus 2 may not be a viable method. Typically, standard 1-L dissolution vessels are used with standard medium volumes (500–1000 mL). The temperature of each vessel is maintained at 37 ± 2 °C. A set of six vessels is used for each test.

Sample Introduction

The dosage form is introduced into the medium using typical sample handling tools such as pipets, spatulas, or weighing boats. Other means of introduction should be well specified, repeatable, and reproducible.

Agitation

Rotation speeds of 50–100 rpm should be used, although the use of other rotation speeds may be warranted.

Sink Conditions

Replenishing medium at various stages of the test may be required to achieve sink conditions. Changing the medium volume at various stages may also be necessary. The exact manner for doing these should be specified.

Sampling Parameters

Sampling time points should be chosen to adequately describe any initial burst release and provide an adequate number of data points for the drug-release profile. Sampling should occur within ± 2 min for time points up to 12 h; it should occur within ± 5 min for time points between 12 and 24 h; and it should occur within ± 30 min for later time points. An accurately determined volume is withdrawn from each vessel, preferably with the paddles

still rotating. The medium may be replenished after sampling.

Dissolution Medium

The medium is chosen by considering the physical and chemical data for the drug substance and dosage unit. See Chapter <1092> for further guidance. The effect of deaeration should be determined. If deaeration affects the dissolution rate, the medium should be degassed using an appropriate technique. Evaporative loss of medium should be minimized. Replenishment of evaporated medium may be necessary for tests of long duration.

Analysis and Calculations

The usual assay for a dissolution sample is HPLC, although other methods, such as spectrophotometry, may be used if feasible. When performing calculations, the analyst should take into consideration all of the dissolution medium adjustments and replenishments.

In Vitro Drug Release Using Apparatus 4

The use of the flow-through cell, Apparatus 4, may be an effective procedure for performing in vitro drug-release testing of some parenteral formulations that are not clear solutions. Typically, the standard tablet cells, 12.0 and 22.6 mm, are used without the tablet clip present, and the unit is operated in the closed-system configuration. The use of other cell designs is allowable. Dissolution medium composition should be selected on the basis of the physical and chemical properties of the drug substance or its API. The flow rate through the cell can also depend on the application. The temperature of the cells is usually maintained at 37 ± 2 °C. A set of six cells is used for each test.

Sample Preparation in the Cells

The standard cell is initially fitted with a 5-mm bead to stop back-flow. One approach can be to add glass beads (typically 1-mm borosilicate glass) to fill the cell up to the intersection of the conical part and the cylindrical part of the cell. Then, an accurately weighed portion of pharmaceutical product to be investigated (e.g., suspension) is added to the cell. Depending on the product and the possibility of aggregation, it may be necessary to disperse the dosage form, typically with more glass beads. The use of additional glass beads to disperse the product also helps to prevent channeling of the fluid through areas of the product (as can occur in a suspension bed), because this can result in non-wetting of portions of the product and a subsequent lack of reproducibility of the data. For nanoparticulate systems, the use of a dialysis sac adapter may be considered. This allows dispersion of the nanoparticulates within

the dialysis sac together with a volume of media as in the “normal” dialysis method discussed above. The media flows around the dialysis sac, which is a preferred dialysis method for nanoparticulates, because it uses a standardized apparatus. Nanoparticulates cannot be suspended directly in the USP Apparatus 4 flow-through cell, because they would either pass through or block the filter material, resulting in sample loss from the flow-through cells and/or system back-pressure and hence failure (7).

Filter Material

The filter head for the flow-through cell should be fitted with an appropriate filter material. The filter material should be durable enough so as to not physically degrade over the duration of the test. Typical filter materials such as glass fiber filters, polyvinylidene fluoride, polytetrafluoroethylene, and regenerated cellulose can be considered. Filter studies for drug adsorption should be performed before testing. If the filter pore size is too small, unacceptable back-pressure can result in the system.

Flow Rate

The flow rate may influence the drug-release results (for diffusion-controlled systems). For this reason, the effect of flow rate on drug release should be examined. Typical flow rates include 4, 8, and 16 mL/min, although other flow rates may be used.

Volume for Drug-Release Testing

The volume of medium used in a closed-loop configuration can vary from as low as 20 mL to several liters. When operating the system in a closed-loop setting, the volume of the medium should be selected with consideration of the solubility of the API and sink conditions. The volume of medium can be adjusted during the same test to achieve a proper concentration. Replacement of medium at various stages of the test may be required to achieve sink conditions. Media replacement may also be conducted as part of the test; an example is changing the media to mimic environmental changes that can occur in vivo (e.g., cellular uptake of liposomes or other nanoparticles) (8).

Sampling Parameters

Sampling time points should be chosen to adequately describe any initial burst release and provide an adequate number of data points for creating the drug-release profile. Sampling should occur within ± 2 min for time points up to 12 h; within ± 5 min for time points between 12 and 24 h; and within ± 30 min for later time points. An accurately determined volume is withdrawn from each media bottle, preferably with the pump still running. It is

important that the medium is mixed adequately during sampling so that a representative sample can be obtained. The medium may be replenished after sampling.

Medium for Drug-Release Testing

When choosing the medium, the analyst should consider the physical and chemical characteristics of the drug substance and dosage unit. See Chapter <1092> for further guidance. The medium should be degassed using an appropriate technique, unless the use of surfactants is necessary. A specified volume of medium should be accurately transferred to each of the six bottles. Media should be stirred throughout the test. Typically, the medium is not heated before or during the test.

Analysis and Calculations

The assay for a dissolution sample is HPLC, although other methods (such as spectrophotometry) may be used if appropriate. Fiber-optic UV probes can be used with USP Apparatus 4. On-line fiber-optic UV probes enable complete characterization of the initial burst release phase, because more data points can be collected in short time intervals. The release profile can be monitored using fiber-optic UV analysis without any interference from the formulation (e.g., microspheres), because the formulation is isolated from the media in USP Apparatus 4.

Typically, release testing of microsphere formulations is performed in a closed-system configuration with USP Apparatus 4. An open system is not practical when long-term testing is required, as with some microsphere products. A cumulative release profile can be obtained in a closed-system configuration by analyzing samples at appropriate time intervals. The change in concentration ($\mu\text{g/mL}$ or mg/mL) determined for each time interval (using an appropriate analytical method), multiplied by the total volume of the dissolution medium, gives the total amount released at different time intervals. The cumulative amount released can be determined in this manner. Calculations should take into account all dissolution medium adjustments and replenishments.

PERFORMANCE TEST FOR SPECIFIC TYPES OF DOSAGE FORMS

Emulsions and Suspensions

For emulsions or suspensions, use either the paddle method (Apparatus 2) or the flow-thru method (Apparatus 4). See Chapter <1092> for further guidance.

Microspheres

The in vitro drug-release test should characterize the mechanism of release and should differentiate between the different release phases of the product.

Sample, separate, and dialysis sac methods have been used conventionally for performance testing of microspheres. However, these methods have the disadvantages of microsphere loss during sampling and aggregation because of improper agitation.

The flow-through cell (USP Apparatus 4) may be used for release testing of microsphere formulations. The microspheres dispersed with the glass beads are packed in the flow-through cells. This modification of the flow-through cell is useful in minimizing aggregation of microspheres and separating them from the release media (9, 10).

The standard tablet cells, 12.0 and 22.6 mm, may be used without the tablet clip, and the unit is operated in the closed-system configuration or the open configuration. Appropriate flow rates can be selected, depending on the formulation and application. The temperature of the cells is usually maintained at 37 ± 2 °C. For accelerated testing, higher temperatures can be used. A set of six cells is used for each test.

Nanoparticles/Nanocrystals

Dissolution of nanoparticle suspensions can be measured by using sampling-separation technique or by monitoring changes in the light-scattering device (11).

Liposomes

Membrane dialysis methods, such as dialysis sac and reverse dialysis sac, are conventionally used for performance testing of liposomes. These methods are needed for separating liposomes from the release media. However, these methods are not based on compendial dissolution/release apparatuses. The procedure and apparatus used for dialysis sac and reverse dialysis sac methods vary among laboratories, and results from different laboratories may not be comparable. Therefore, a dialysis method based on a compendial dissolution/release apparatus may be more appropriate for performance testing of liposomes.

USP Apparatus 4, using a flow-through cell fitted with a dialysis adapter, may be used for performance testing of liposome formulations. An adapter has been designed for the 22.6-mm diameter flow-through cell. A dialysis membrane is placed over the adapter, and this assembly is then placed over the conical part of the flow-through cell. The method has been shown to be superior to dialysis and reverse dialysis sac methods for liposomes containing the hydrophobic drug dexamethasone in terms of reproducibility and discriminatory ability (7).

The flow-through cell (USP Apparatus 4), fitted with a dialysis adapter, may be used for performance testing of liposome formulations. However, if placed directly in the flow-through cell, the liposomes (nanometer size range) may either block the filter or pass through it. The dialysis adapter may be used with 12- or 22.6-mm diameter flow-through cells. Flow-through cell size may be selected based upon the drug concentration in the formulation and the volume of formulation to be used for release testing. Higher volumes can be used with the 22.6-mm diameter flow-through cell.

The dialysis adapter framework consists of a circular top and bottom supported by three wires of a suitable material (such as peek, metal, or others). The circular top has an opening for sample introduction, and this opening can be closed with a leak-proof screw. A dialysis membrane/bag is placed over this adapter frame and sealed with "O" rings. This assembly is then placed over the conical part of the flow-through cell. This method offers several advantages: (1) uniform and adequate agitation outside the dialysis bag; (2) uniform temperature in the sample cell per USP requirements; and (3) low variation among the replicates (7).

The standard tablet cells, 12.0 and 22.6 mm, may be used without the tablet clip present, and the unit may be operated in the open- or closed-system configuration. Appropriate flow rates can be selected, depending on the formulation and application. The temperature of the cells is usually maintained at 37 ± 2 °C. A set of six cells may be used for each test.

Implants

The flow-through cell (USP Apparatus 4) may be used for release testing of implant formulations (12). The implants may be held in the flow-through cell with a special holder. The standard tablet cells, 12.0 and 22.6 mm, may be used without the tablet clip when the unit is operated in the closed-system configuration or in the open configuration. Appropriate flow rates can be selected, depending on the formulation and application. The temperature of the cells is usually maintained at 37 ± 2 °C. For accelerated testing, higher temperatures can be used. A set of six cells is used for each test.

Drug-Eluting Stents

USP Apparatus 4 and 7 with stent holders may be used for performance testing of drug-eluting stents. Small volumes of release media can be used with both of these systems (about 15 and 4 mL for USP Apparatus 4 and 7, respectively).

Small-volume dissolution apparatuses, with volumes of 1–4 mL, have been used to measure drug release from medical devices, such as drug-eluting stents. The instrument features a magnetically driven reciprocation mechanism and heater jackets to determine the rate of drug release from stents.

In Situ Forming Gels

In situ forming gels are very challenging because of problems associated with gelling in vitro; a suitable depot must be formed without emulsification or sample dispersion occurring. USP Apparatus 2 and 4 have been used. Most in vitro release tests have been performed using variants of the sample-and-separate methodology. In addition, the dialysis method has also been used to evaluate in situ depot-forming formulations. When performing an in vitro release test, the pre-gelled formulation is usually held in a special retainer to achieve a defined geometry or area of formulation–buffer interface. Alternatively, the already formed formulation can be placed into the release medium using a syringe (13).

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