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⟨1228.1⟩ DRY HEAT DEPYROGENATION

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INTRODUCTION

Dry heat is the method most frequently used for the depyrogenation of heat stable materials. Dry heat depyrogenation is dependent upon two parameters: time and temperature, which equates to a thermal input. As a result, dry heat depyrogenation processes can be easily monitored/controlled and are highly reproducible. Depyrogenation processes typically operate at a range of temperatures from approximately 170° up to about 400°.

The most prevalent pyrogenic agents in ▲parenteral▲ (ERR 1-Mar-2024) manufacturing that are of concern relative to patient safety are bacterial endotoxins, found in the outer cell walls of Gram-negative bacteria. The destruction of bacterial endotoxins (depyrogenation) by dry heat has been studied extensively and has been shown to follow first order kinetics. The well-defined kinetics of inactivation makes it possible to predict the efficacy of dry heat processes operating at different times and temperatures by understanding the total thermal input (F_D).

The range of temperatures used for dry heat depyrogenation overlaps the upper range of temperatures used for dry heat sterilization (see *Dry Heat Sterilization* ⟨1229.8⟩). This is because bacterial endotoxins are more resistant to the effects of dry heat than the most heat-resistant bacterial spores. This chapter provides an overview of the process of dry heat depyrogenation, its control, and validation.

TECHNOLOGIES USED FOR DEPYROGENATION BY DRY HEAT

Although all dry heat depyrogenation processes rely strictly on time of exposure and temperature to assure effectiveness, the equipment used typically falls into two categories: the dry heat “batch” oven and continuous tunnel systems. Batch ovens are routinely used for the depyrogenation of product containers, most often glass, but also other heat stable product contact parts or laboratory equipment. Continuous tunnels, on the other hand, are used primarily to depyrogenate glass product containers.

Batch Ovens

Circulating heated air is used to heat the load items, which may be individually covered or wrapped in a material that is unaffected by the temperature used, or placed in a lidded container for protection during pre- and post-process handling. When depyrogenation and sterilization are to be achieved in the same process, air supplied to the oven is passed through one or more high efficiency particulate air (HEPA) filter(s) to maintain sterility within the oven after completion of the dwell period. These forced air ovens typically operate at a positive air pressure differential relative to the surrounding room. This design results in particulate air quality that can meet ISO 5 requirements to reduce particulate matter and microbial contamination risk throughout processing.

In order to ensure sufficient lethality and process control, oven control probe(s) must maintain a predefined temperature for a predefined time period prior to cooling. The limited heat transfer capacity of air requires that items in the oven be placed in fixed locations confirmed acceptable during the cycle development/validation effort. Caution should be exercised in defining variable load patterns as minimum load sizes may result in inadvertent slower heating of the load and greater temperature variability. Smaller facilities may use a single door oven, but the principles of operation and validation are the same as with larger double door production units. The important batch oven process variables are set-point temperature, duration of dwell period, load type and configuration, airflow characteristics, and container size.

Continuous Tunnels

The use of tunnels for dry heat depyrogenation of glass containers on a moving conveyor allows for substantially higher throughput and packing densities than the batch process, reduces handling, and is ordinarily integrated with a washing and filling system. Tunnels typically use forced heated air systems or radiant IR systems that recirculate air through a battery of HEPA filters. Load items in tunnels are typically fed directly from an integrated container washing system.

Depyrogenation tunnels have separate zones for heating and cooling, allowing for continuous in-feed and discharge at temperatures appropriate for production purposes. The tunnel is maintained at constant airflow and temperature conditions during use, and as glass passes through the tunnel it is heated to depyrogenating temperatures and cooled before exiting. Although the conditions within the tunnel are essentially constant and well controlled, the temperature of the glass as it passes through the tunnel on the conveyor will change with its location. Dwell time is controlled by adjusting the conveyor speed,[1] which in the depyrogenation tunnel is the process parameter that governs exposure time.

The air in the tunnel is most commonly heated using electrical coils but other heat sources, such as infrared or high-pressure steam, have been used. For energy conservation, heated air in depyrogenation tunnels is often recirculated. The important continuous tunnel process

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variables are heating zone temperature, cooling zone temperature, belt speed, and container mass per unit.

DRY HEAT DEPYROGENATION FUNDAMENTALS

Dry heat depyrogenation uses air first to heat and then to cool the items. The limited heat capacity of dry air results in relatively slow heating and cooling of the load items. Variability in temperature distribution in dry heat ovens and tunnels is typically much higher than that observed in moist heat systems. The limited heat capacity of air requires that items in ovens be placed in the same locations as confirmed acceptable in the cycle development/validation effort. Packing and thermal mass will also play critical roles in temperature management. Caution must also be exercised with varying load mass and distribution as in some instances (resulting from oven design, air flow characteristics, and control probe position) minimum load sizes may result in process variability.

DEPYROGENATION PROCESS CONTROL

Process temperatures in dry heat depyrogenation are controlled by calibrated temperature sensors placed at specific locations within the equipment. The exposure portion of the process is designed to attain a minimum dwell time at a predefined minimum temperature ensuring that depyrogenation conditions are adequately uniform. The defined dwell time is determined by using measurement devices (e.g., thermocouples) directly in contact with the items to be depyrogenated during development. The inactivation of bacterial endotoxins by dry heat involves the control of only two parameters: time and temperature.

The simplicity of process control for these parameters provides a predictable depyrogenation effect. Once validated, a clear understanding of the inputs to the system, meaning resident endotoxin load on incoming materials, and outputs of the system, meaning reduction of resident levels of endotoxin to safe levels, is more meaningful than spiking with unnaturally high levels of challenge material and looking for a prescriptive requirement for log reduction. The dosimetric measurement for dry heat depyrogenation processes is the F_D unit. An $F_D = 1$ is defined as the depyrogenation effect achieved by 1 min of heating at 250°. The F_D-value enables the integration of temperature over the process duration (time). By convention, the rate at which depyrogenation destruction rate (*D*-value) varies as a function of temperature change is defined as the *z*-value. The *z*-value for dry heat depyrogenation has been shown to be in the range of 45°–55°. For the purposes of this chapter, 50° is used as a standard *z*-value. Other values may be used. (1,2)

The F_D approach is used as a means to compare dry heat depyrogenation effects produced by processes that operate at varying temperatures. Basic mathematics can be used to calculate the depyrogenation effect produced at temperatures other than 250° to determine equivalence to that provided at 250°.

Using a reference temperature of 250° and an assumed *z*-value of 50°, the F_D calculation can be determined:

$$F_D = \int_{t_1}^{t_2} 10^{\left(\frac{T-250}{50}\right)} dt = \sum_{t_1}^{t_2} 10^{\left(\frac{T-250}{50}\right)} \Delta t$$

F_D = accumulated destruction

t_1 = process start time

t_2 = process end time

T = temperature at each time increment

Δt = time interval between temperature measurements

Summing the instantaneous temperature contributions over the entire depyrogenation process allows for the calculation of the overall process efficacy or F_D delivered over the course of the process. Many commercial data loggers are equipped with software that enables them to make this calculation and integrate the total F_D accumulated during a process. The F_D calculation is used during initial validation, validation maintenance, and change control. The mathematical principles of the F_D calculation are essentially the same as those used to calculate lethality (F_0) values in moist heat sterilization. F_D values are used to confirm process consistency over time as correlation to endotoxin destruction is rarely possible.

VALIDATION

Because dry heat depyrogenation is appropriate only for heat stable materials, a high margin for safety is always attainable. Times and temperatures used for the purpose of destroying challenge materials can result in extreme challenges to material integrity and stability. Attention to depyrogenation processes, including an understanding of the resident endotoxin load on incoming materials and reduction to levels needed to assure patient safety, should take place during drug product development, prior to validation.

Equipment Qualification

Equipment Qualification (EQ) is a predefined program that focuses on the processing equipment to confirm that it has been properly installed and operates as intended prior to evaluation of the process. In some companies, EQ may be separated into installation qualification (IQ) and operational qualification (OQ), or combined together under a joint terminology of installation/operational qualification (I/OQ). Equipment qualification provides a baseline for preventive maintenance and change control assuring reproducibility of equipment operation over time.

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Empty Chamber Temperature Distribution for Ovens

The oven should be evaluated for empty chamber temperature distribution. This is assessed by measurement of temperature at each corner of oven, near the controlling probe(s) and other locations as justified. Differences in the cycle dwell period can be discounted in this evaluation, as only the shortest dwell period need be evaluated. The evaluation is best performed over the last few minutes of the dwell period once the system has fully stabilized. The acceptance criteria for this test vary with the oven's design and operating conditions; however, temperature distribution is typically substantially less uniform than observed in autoclaves and may be ±15° or more. Depyrogenation ovens that are located at floor level may have even greater ranges in temperature. The temperature distribution measurement may be of value in the evaluation of changes to the oven.

Empty Temperature Distribution in Tunnels

While these studies are often done, they are actually of limited value. Unloaded depyrogenation tunnels will always produce far more variability in temperature distribution than will a fully loaded tunnel. Therefore, for depyrogenation tunnels, temperature studies under fully loaded conditions only are indicated. Important to the proper operation of the dry heat tunnel is the establishment of the required air flow balance between the tunnel and the adjoining areas. Improper air flow can cause uneven heating across the load being processed. The temperature distribution measurement may be of value in the evaluation of changes to the tunnel.

Component Mapping

The ability of dry heat to penetrate load items and to bring them to the required temperature should be determined.

Load items that are complex, of significant mass, with enclosed volumes and product contact surfaces that must be depyrogenated, should be subjected to component mapping to determine internal cold spots. All load items should be prepared, wrapped (if that is the practice), and oriented in a manner consistent with how they will be processed. Glass typically enters the depyrogenation process (whether tunnel or oven) wet and must be evaluated wet to properly determine the effect on overall thermal input. Mapping of glass components to be processed in tunnels is not necessary; all monitoring of temperature in tunnels is accomplished with probes in contact with the bottom of the container.

Load Mapping in Ovens

Fixed loading patterns are necessary in oven depyrogenation because of the limited heat capacity of the air; fully packed conditions because of their greater mass ordinarily result in the best process temperature uniformity. Load mapping assures that items placed throughout the load attain the desired depyrogenation conditions. Identification of cold zones within the oven should be established during depyrogenation cycle development. Information from the load mapping is used to adjust cycle timing to assure appropriate efficacy across the entire load. It may be possible to validate maximum and minimum loads (as determined by either the number of items or their mass).

F_D is calculated from the temperature data at all monitored locations within the load pattern.

Load Mapping in Tunnels

Load mapping can be assessed using sets of calibrated sensors (i.e., trailing or wireless temperature sensors) positioned within the glass pack as it moves through the tunnel. Temperature sensors should be placed into direct contact with the glass item at the bottom of the container. Temperature measurements should be made on the leading edge, the middle (highest density), and the trailing edge of the glass pack across the width of the conveyor belt. There should be NLT 5 temperature sensors positioned across the belt in each section of the load. F_D is calculated from the temperature data at all monitored locations. Studies should be performed using all container sizes to determine the lowest F_D locations. The F_D results can be used to support the selection of containers/conditions to be evaluated in the confirmation studies (see below). There is no requirement to perform temperature heat distribution measurements during these studies.

Confirmation of Depyrogenation

The materials/glass components to be depyrogenated should be assessed for their incoming endotoxin content prior to the validation study. This would include glass as received and immediately after washing. All tested materials should be handled and prepared using defined procedures. Materials and glass prepared in the same manner are used in the depyrogenation validation studies. The addition of challenge material to the load items, including a requirement to demonstrate a 3-log reduction, may not be required if time and temperature studies consistently indicate that depyrogenation conditions are met. Temperature monitoring as described above must be done simultaneously with the depyrogenation confirmation studies. The confirmation studies should be performed at reduced time-temperature conditions from those utilized in routine processing and deliver lower F_D results when compared to those determined in the mapping studies and are considered “worst case” confirmation of depyrogenation process efficacy. There is no requirement to perform temperature heat distribution measurements during these studies.

OVENS

A minimum of five (5) samples should be taken in proximity at NLT 10 temperature-monitored locations (including those determined to be the coldest from the oven load mapping study) in the oven and tested for endotoxin content post-processing. The process is considered acceptable if the amount of endotoxin per sample is NMT 0.1 EU.

TUNNELS

A minimum of five (5) samples should be taken in proximity to each monitored position within the tunnel load (including those determined to be the coldest from the tunnel load mapping study) and tested for endotoxin content post-processing. The process is

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considered acceptable if the amount of endotoxin per sample is NMT 0.1 EU.

ROUTINE PROCESS CONTROL

As with all processes, after the dry heat depyrogenation process has been validated, it must be subject to ongoing controls that maintain it within the validated state at all times. Temperature and exposure time, which are the important dry heat depyrogenation parameters, can be used to confirm performance on a routine basis. Where direct assessment of F_D is not possible, assuring that the temperature and exposure time conditions were met results in an equivalent confidence that the depyrogenation system operated in a validated state of control.

Depyrogenation ⟨1228⟩ details the general practices that are appropriate for all depyrogenation systems. This is accomplished by a number of related practices that are essential for the continued use of the process over an extended period of time. The essential practices to maintain validated status include calibration, physical measurements, periodic endotoxin assessment on incoming materials, ongoing process control, change control, preventive maintenance, and periodic reassessment and training.

APPENDIX

Additional Sources of Information

- Tsuji K, Harrison S. Dry heat destruction of lipopolysaccharide: dry heat destruction kinetics. *Appl Environ Microbio.* 1978; 36(5):710–714.
- Ludwig J., Avis KE. Dry heat inactivation of endotoxin on the surface of glass. *J Parenteral Sci Technol.* 1990; 44(1):4–12.
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1. Tsuji K, Lewis A. Dry heat destruction of lipopolysaccharide: a mathematical approach to process evaluation. *Appl Environ Microbio.* 1978; 36(5):715–719.
2. Akers MJ, Ketron KM, Thompson, BR. F value requirements for the destruction of endotoxin in the validation of dry-heat sterilization/depyrogenation cycles. *J Parenteral Sci Technol.* 1982; (36):12–6.

[1] Not all tunnels have a variable speed capacity.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
<1228.1> DRY HEAT DEPYROGENATION	Leslie Furr Associate Scientific Liaison	GCM2022 General Chapters - Microbiology 2022

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