GUIDE TO INSPECTIONS OF LYOPHILIZATION OF PARENTERALS

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INTRODUCTION

Lyophilization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. The process consists of three separate, unique, and interdependent processes; freezing, primary drying (sublimation), and secondary drying (desorption).

The advantages of lyophilization include:

Ease of processing a liquid, which simplifies aseptic handling

Enhanced stability of a dry powder

Removal of water without excessive heating of the product

Enhanced product stability in a dry state

Rapid and easy dissolution of reconstituted product

Disadvantages of lyophilization include:

Increased handling and processing time

Need for sterile diluent upon reconstitution

Cost and complexity of equipment

The lyophilization process generally includes the following steps:

o Dissolving the drug and excipients in a suitable solvent, generally water for injection (WFI).

o Sterilizing the bulk solution by passing it through a 0.22 micron bacteria-retentive filter.

o Filling into individual sterile containers and partially stoppering the containers under aseptic conditions.

o Transporting the partially stoppered containers to the lyophilizer and loading into the chamber under aseptic conditions.

o Freezing the solution by placing the partially stoppered containers on cooled shelves in a freeze-drying chamber or pre-freezing in another chamber.

o Applying a vacuum to the chamber and heating the shelves in order to evaporate the water from the frozen state.

o Complete stoppering of the vials usually by hydraulic or screw rod stoppering mechanisms installed in the lyophilizers.

There are many new parenteral products, including anti-infectives, biotechnology derived products, and in-vitro diagnostics which are manufactured as lyophilized products. Additionally, inspections have disclosed potency, sterility and stability problems associated with the manufacture and control of lyophilized products. In order to provide guidance and information to investigators, some industry procedures and deficiencies associated with lyophilized products are identified in this Inspection Guide.

It is recognized that there is complex technology associated with the manufacture and control of a lyophilized pharmaceutical dosage form. Some of the important aspects of these operations include: the formulation of solutions; filling of vials and validation of the filling operation; sterilization and engineering aspects of the lyophilizer; scale-up and validation of the lyophilization cycle; and testing of the end product. This discussion will address some of the problems associated with the manufacture and control of a lyophilized dosage form.

PRODUCT TYPE/FORMULATION

Products are manufactured in the lyophilized form due to their instability when in solution. Many of the antibiotics, such as some of the semi-synthetic penicillins, cephalosporins, and also some of the salts of erythromycin, doxycycline and chloramphenicol are made by the lyophilization process. Because they are antibiotics, low bioburden of these formulations would be expected at the time of batching. However, some of the other dosage forms that are lyophilized, such as hydrocortisone sodium succinate, methylprednisolone sodium succinate and many of the biotechnology derived products, have no antibacterial effect when in solution.

For these types of products, bioburden should be minimal and the bioburden should be determined prior to sterilization of these bulk solutions prior to filling. Obviously, the batching or compounding of these bulk solutions should be controlled in order to prevent any potential increase in microbiological levels that may occur up to the time that the bulk solutions are filtered (sterilized). The concern with any microbiological level is the possible increase in endotoxins that may develop. Good practice for the compounding of lyophilized products would also include batching in a controlled environment and in sealed tanks, particularly if the solution is to be held for any length of time prior to sterilization.

In some cases, manufacturers have performed bioburden testing on bulk solutions after prefiltration and prior to final filtration. While the testing of such solutions may be meaningful in determining the bioburden for sterilization, it does not provide any information regarding the potential formation or presence of endotoxins. While the testing of 0.1 ml samples by LAL methods of bulk solution for endotoxins is of value, testing of at least 100 ml size samples prior to prefiltration, particularly for the presence of gram negative organisms, would be of greater value in evaluating the process. For example, the presence of Pseudomonas sp. in the bioburden of a bulk solution has been identified as an objectionable condition.

FILLING

The filling of vials that are to be lyophilized has some problems that are somewhat unique. The stopper is placed on top of the vial and is ultimately seated in the lyophilizer. As a result the contents of the vial are subject to contamination until they are actually sealed.

Validation of filling operations should include media fills and the sampling of critical surfaces and air

during active filling (dynamic conditions).

Because of the active involvement of people in filling and aseptic manipulations, an environmental program should also include an evaluation of microbiological levels on people working in aseptic processing areas. One method of evaluation of the training of operators working in aseptic processing facilities includes the surface monitoring of gloves and/or gowns on a daily basis. Manufacturers are actively sampling the surfaces of personnel working in aseptic processing areas. A reference which provides for this type of monitoring is the USP XXII discussion of the Interpretation of Sterility Test Results. It states under the heading of "Interpretation of Quality Control Tests" that review consideration should be paid to environmental control data, including...microbial monitoring, records of operators, gowns, gloves, and garbing practices. In those situations in which manufacturers have failed to perform some type of personnel monitoring, or monitoring has shown unacceptable levels of contamination, regulatory situations have resulted.

Typically, vials to be lyophilized are partially stoppered by machine. However, some filling lines have been noted which utilize an operator to place each stopper on top of the vial by hand. At this time, it would seem that it would be difficult for a manufacturer to justify a hand-stoppering operation, even if sterile forceps are employed, in any type of operation other than filling a clinical batch or very small number of units. Significant regulatory situations have resulted when some manufacturers have hand-stoppered vials. Again, the concern is the immediate avenue of contamination offered by the operator. It is well recognized that people are the major source of contamination in an aseptic processing filling operation. The longer a person works in an aseptic operation, the more microorganisms will be shed and the greater the probability of contamination.

Once filled and partially stoppered, vials are transported and loaded into the lyophilizer. The transfer and handling, such as loading of the lyophilizer, should take place under primary barriers, such as the laminar flow hoods under which the vials were filled. Validation of this handling should also include the use media fills.

Regarding the filling of sterile media, there are some manufacturers who carry out a partial lyophilization cycle and freeze the media. While this could seem to greater mimic the process, the freezing of media could reduce microbial levels of some contaminants. Since the purpose of the media fill is to evaluate and justify the aseptic capabilities of the process, the people and the system, the possible reduction of microbiological levels after aseptic manipulation by freezing would not be warranted. The purpose of a media fill is not to determine the lethality of freezing and its effect on any microbial contaminants that might be present.

In an effort to identify the particular sections of filling and aseptic manipulation that might introduce contamination, several manufacturers have resorted to expanded media fills. That is, they have filled approximately 9000 vials during a media fill and segmented the fill into three stages. One stage has included filling of 3000 vials and stoppering on line; another stage included filling 3000 vials, transportation to the lyophilizer and then stoppering; a third stage included the filling of 3000 vials, loading in the lyophilizer, and exposure to a portion of the nitrogen flush and then stoppering. Since lyophilizer sterilization and sterilization of the nitrogen system used to backfill require separate validation, media fills should primarily validate the filling, transportation and loading aseptic operations.

The question of the number of units needed for media fills when the capacity of the process is less than 3000 units is frequently asked, particularly for clinical products. Again, the purpose of the media fill is to assure that product can be aseptically processed without contamination under operating conditions. It would seem, therefore, that the maximum number of units of media filled be equivalent to the maximum batch size if it is less than 3000 units.

After filling, dosage units are transported to the lyophilizer by metal trays. Usually, the bottom of the trays are removed after the dosage units are loaded into the lyophilizer. Thus, the dosage units lie directly on the lyophilizer shelf. There have been some situations in which manufacturers have loaded the dosage units on metal trays which were not removed. Unfortunately, at one manufacturer, the trays warped which caused a moisture problem in some dosage units in a batch.

In the transport of vials to the lyophilizer, since they are not sealed, there is concern for the potential for contamination. During inspections and in the review of new facilities, the failure to provide laminar flow coverage or a primary barrier for the transport and loading areas of a lyophilizer has been regarded as an objectionable condition. One manufacturer as a means of correction developed a laminar flow cart to transport the vials from the filling line to the lyophilizer. Other manufacturers building new facilities have located the filling line close to the lyophilizer and have provided a primary barrier extending from the filling line to the lyophilizer.

In order to correct this type of problem, another manufacturer installed a vertical laminar flow hood between the filling line and lyophilizer. Initially, high velocities with inadequate return caused a contamination problem in a media fill. It was speculated that new air currents resulted in rebound contamination off the floor. Fortunately, media fills and smoke studies provided enough meaningful information that the problem could be corrected prior to the manufacture of product. Typically, the lyophilization process includes the stoppering of vials in the chamber.

Another major concern with the filling operation is assurance of fill volumes. Obviously, a low fill would represent a subpotency in the vial. Unlike a powder or liquid fill, a low fill would not be readily apparent after lyophilization particularly for a biopharmaceutical drug product where the active ingredient may be only a milligram. Because of the clinical significance, sub-potency in a vial potentially can be a very serious situation.

For example, in the inspection of a lyophilization filling operation, it was noted that the firm was having a filling problem. The gate on the filling line was not coordinated with the filling syringes, and splashing and partial filling was occurring. It was also observed that some of the partially filled vials were loaded into the lyophilizer. This resulted in rejection of the batch.

On occasion, it has been seen that production operators monitoring fill volumes record these fill volumes only after adjustments are made. Therefore, good practice and a good quality assurance program would include the frequent monitoring of the volume of fill, such as every 15 minutes. Good practice would also include provisions for the isolation of particular sections of filling operations when low or high fills are encountered.

There are some atypical filling operations which have not been discussed. For example, there have also been some situations in which lyophilization is performed on trays of solution rather than in vials. Based on the current technology available, it would seem that for a sterile product, it would be difficult to justify this procedure.

The dual chamber vial also presents additional requirements for aseptic manipulations. Media fills should include the filling of media in both chambers. Also, the diluent in these vials should contain a preservative. (Without a preservative, the filling of diluent would be analogous to the filling of media. In such cases, a 0% level of contamination would be expected.)

LYOPHILIZATION CYCLE AND CONTROLS

After sterilization of the lyophilizer and aseptic loading, the initial step is freezing the solution. In some cycles, the shelves are at the temperature needed for freezing, while for other cycles, the product is loaded and then the shelves are taken to the freezing temperature necessary for product

freeze. In those cycles in which the shelves are precooled prior to loading, there is concern for any ice formation on shelves prior to loading. Ice on shelves prior to loading can cause partial or complete stoppering of vials prior to lyophilization of the product. A recent field complaint of a product in solution and not lyophilized was attributed to preliminary stoppering of a few vials prior to exposure to the lyophilization cycle. Unfortunately, the firm's 100% vial inspection failed to identify the defective vial.

Typically, the product is frozen at a temperature well below the eutectic point.

The scale-up and change of lyophilization cycles, including the freezing procedures, have presented some problems. Studies have shown the rate and manner of freezing may affect the quality of the lyophilized product. For example, slow freezing leads to the formation of larger ice crystals. This results in relatively large voids, which aid in the escape of water vapor during sublimation. On the other hand, slow freezing can increase concentration shifts of components. Also, the rate and manner of freezing has been shown to have an affect on the physical form (polymorph) of the drug substance.

It is desirable after freezing and during primary drying to hold the drying temperature (in the product) at least 4-5o below the eutectic point. Obviously, the manufacturer should know the eutectic point and have the necessary instrumentation to assure the uniformity of product temperatures. The lyophilizer should also have the necessary instrumentation to control and record the key process parameters. These include: shelf temperature, product temperature, condenser temperature, chamber pressure and condenser pressure. The manufacturing directions should provide for time, temperature and pressure limits necessary for a lyophilization cycle for a product. The monitoring of product temperature is particularly important for those cycles for which there are atypical operating procedures, such as power failures or equipment breakdown.

Electromechanical control of a lyophilization cycle has utilized cam-type recorder-controllers. However, newer units provide for microcomputer control of the freeze drying process. A very basic requirement for a computer controlled process is a flow chart or logic. Typically, operator involvement in a computer controlled lyophilization cycle primarily occurs at the beginning. It consists of loading the chamber, inserting temperature probes in product vials, and entering cycle parameters such as shelf temperature for freezing, product freeze temperature, freezing soak time, primary drying shelf temperature and cabinet pressure, product temperature for establishment of fill vacuum, secondary drying shelf temperature, and secondary drying time.

In some cases, manufacturers have had to continuously make adjustments in cycles as they were being run. In these situations, the lyophilization process was found to be non-validated.

Validation of the software program of a lyophilizer follows the same criteria as that for other processes. Basic concerns include software development, modifications and security. The Guide to Inspection of Computerized Systems in Drug Processing contains a discussion on potential problem areas relating to computer systems. A Guide to the Inspection of Software Development Activities is a reference that provides a more detailed review of software requirements.

Leakage into a lyophilizer may originate from various sources. As in any vacuum chamber, leakage can occur from the atmosphere into the vessel itself. Other sources are media employed within the system to perform the lyophilizing task. These would be the thermal fluid circulated through the shelves for product heating and cooling, the refrigerant employed inside the vapor condenser cooling surface and oil vapors that may migrate back from the vacuum pumping system.

Any one, or a combination of all, can contribute to the leakage of gases and vapors into the system. It is necessary to monitor the leak rate periodically to maintain the integrity of the system. It is also necessary, should the leak rate exceed specified limits, to determine the actual leak site for purposes

of repair.

Thus, it would be beneficial to perform a leak test at some time after sterilization, possibly at the beginning of the cycle or prior to stoppering. The time and frequency for performing the leak test will vary and will depend on the data developed during the cycle validation. The pressure rise found acceptable at validation should be used to determine the acceptable pressure rise during production. A limit and what action is to be taken if excessive leakage is found should be addressed in some type of operating document.

In order to minimize oil vapor migration, some lyophilizers are designed with a tortuous path between the vacuum pump and chamber. For example, one fabricator installed an oil trap in the line between the vacuum pump and chamber in a lyophilizer with an internal condenser. Leakage can also be identified by sampling surfaces in the chamber after lyophilization for contaminants. One could conclude that if contamination is found on a chamber surface after lyophilization, then dosage units in the chamber could also be contaminated. It is a good practice as part of the validation of cleaning of the lyophilization chamber to sample the surfaces both before and after cleaning.

Because of the lengthy cycle runs and strain on machinery, it is not unusual to see equipment malfunction or fail during a lyophilization cycle. There should be provisions in place for the corrective action to be taken when these atypical situations occur. In addition to documentation of the malfunction, there should be an evaluation of the possible effects on the product (e.g., partial or complete meltback. Refer to subsequent discussion). Merely testing samples after the lyophilization cycle is concluded may be insufficient to justify the release of the remaining units. For example, the leakage of chamber shelf fluid into the chamber or a break in sterility would be cause for rejection of the batch.

The review of Preventive Maintenance Logs, as well as Quality Assurance Alert Notices, Discrepancy Reports, and Investigation Reports will help to identify problem batches when there are equipment malfunctions or power failures. It is recommended that these records be reviewed early in the inspection.

CYCLE VALIDATION

Many manufacturers file (in applications) their normal lyophilization cycles and validate the lyophilization process based on these cycles. Unfortunately, such data would be of little value to substantiate shorter or abnormal cycles. In some cases, manufacturers are unaware of the eutectic point. It would be difficult for a manufacturer to evaluate partial or abnormal cycles without knowing the eutectic point and the cycle parameters needed to facilitate primary drying.

Scale-up for the lyophilized product requires a knowledge of the many variables that may have an effect on the product. Some of the variables would include freezing rate and temperature ramping rate. As with the scale-up of other drug products, there should be a development report that discusses the process and logic for the cycle. Probably more so than any other product, scale-up of the lyophilization cycle is very difficult.

There are some manufacturers that market multiple strengths, vial sizes and have different batch sizes. It is conceivable and probable that each will have its own cycle parameters. A manufacturer that has one cycle for multiple strengths of the same product probably has done a poor job of developing the cycle and probably has not adequately validated their process. Investigators should review the reports and data that support the filed lyophilization cycle.

LYOPHILIZER STERILIZATION/DESIGN

The sterilization of the lyophilizer is one of the more frequently encountered problems noted during inspections. Some of the older lyophilizers cannot tolerate steam under pressure, and sterilization is marginal at best. These lyophilizers can only have their inside surfaces wiped with a chemical agent that may be a sterilant but usually has been found to be a sanitizing agent. Unfortunately, piping such as that for the administration of inert gas (usually nitrogen) and sterile air for backfill or vacuum break is often inaccessible to such surface "sterilization" or treatment. It would seem very difficult for a manufacturer to be able to demonstrate satisfactory validation of sterilization of a lyophilizer by chemical "treatment".

Another method of sterilization that has been practiced is the use of gaseous ethylene oxide. As with any ethylene oxide treatment, humidification is necessary. Providing a method for introducing the sterile moisture with uniformity has been found to be difficult.

A manufacturer has been observed employing Water For Injection as a final wash or rinse of the lyophilizer. While the chamber was wet, it was then ethylene oxide gas sterilized. As discussed above, this may be satisfactory for the chamber but inadequate for associated plumbing.

Another problem associated with ethylene oxide is the residue. One manufacturer had a common ethylene oxide/nitrogen supply line to a number of lyophilizers connected in parallel to the system. Thus, there could be some ethylene oxide in the nitrogen supply line during the backfilling step. Obviously, this type of system is objectionable.

A generally recognized acceptable method of sterilizing the lyophilizer is through the use of moist steam under pressure. Sterilization procedures should parallel that of an autoclave, and a typical system should include two independent temperature sensing systems. One would be used to control and record temperatures of the cycle as with sterilizers, and the other would be in the cold spot of the chamber. As with autoclaves, lyophilizers should have drains with atmospheric breaks to prevent back siphonage.

As discussed, there should also be provisions for sterilizing the inert gas or air and the supply lines. Some manufacturers have chosen to locate the sterilizing filters in a port of the chamber. The port is steam sterilized when the chamber is sterilized, and then the sterilizing filter, previously sterilized, is aseptically connected to the chamber. Some manufacturers have chosen to sterilize the filter and downstream piping to the chamber in place. Typical sterilization-in-place of filters may require steaming of both to obtain sufficient temperatures. In this type of system, there should be provisions for removing and/or draining condensate. The failure to sterilize nitrogen and air filters and the piping downstream leading into the chamber has been identified as a problem on a number of inspections.

Since these filters are used to sterilize inert gas and/or air, there should be some assurance of their integrity. Some inspections have disclosed a lack of integrity testing of the inert gas and/or air filter. The question is frequently asked how often should the vent filter be tested for integrity? As with many decisions made by manufacturers, there is a level of risk associated with the operation, process or system, which only the manufacturer can decide. If the sterilizing filter is found to pass the integrity test after several uses or batches, then one could claim its integrity for the previous batches. However, if it is only tested after several batches have been processed and if found to fail the integrity test, then one could question the sterility of all of the previous batches processed. In an effort to minimize this risk, some manufacturers have resorted to redundant filtration.

For most cycles, stoppering occurs within the lyophilizer. Typically, the lyophilizer has some type of rod or rods (ram) which enter the immediate chamber at the time of stoppering. Once the rod enters the chamber, there is the potential for contamination of the chamber. However, since the vials are stoppered, there is no avenue for contamination of the vials in the chamber which are now stoppered. Generally, lyophilizers should be sterilized after each cycle because of the potential for contamination

of the shelf support rods. Additionally, the physical act of removing vials and cleaning the chamber can increase levels of contamination.

In some of the larger units, the shelves are collapsed after sterilization to facilitate loading. Obviously, the portions of the ram entering the chamber to collapse the shelves enters from a non-sterile area. Attempts to minimize contamination have included wiping the ram with a sanitizing agent prior to loading. Control aspects have included testing the ram for microbiological contamination, testing it for residues of hydraulic fluid, and testing the fluid for its bacteriostatic effectiveness. One lyophilizer fabricator has proposed developing a flexible "skirt" to cover the ram.

In addition to microbiological concerns with hydraulic fluid, there is also the concern with product contamination.

During steam sterilization of the chamber, there should be space between shelves that permit passage of free flowing steam. Some manufacturers have placed "spacers" between shelves to prevent their total collapse. Others have resorted to a two phase sterilization of the chamber. The initial phase provides for sterilization of the shelves when they are separated. The second phase provides for sterilization of the chamber and piston with the shelves collapsed.

Typically, biological indicators are used in lyophilizers to validate the steam sterilization cycle. One manufacturer of a Biopharmaceutical product was found to have a positive biological indicator after sterilization at 121oC for 45 minutes. During the chamber sterilization, trays used to transport vials from the filling line to the chamber were also sterilized. The trays were sterilized in an inverted position on shelves in the chamber. It is believed that the positive biological indicator is the result of poor steam penetration under these trays.

The sterilization of condensers is also a major issue that warrants discussion. Most of the newer units provide for the capability of sterilization of the condenser along with the chamber, even if the condenser is external to the chamber. This provides a greater assurance of sterility, particularly in those situations in which there is some equipment malfunction and the vacuum in the chamber is deeper than in the condenser.

Malfunctions that can occur, which would indicate that sterilization of the condenser is warranted, include vacuum pump breakdown, refrigeration system failures and the potential for contamination by the large valve between the condenser and chamber. This is particularly true for those units that have separate vacuum pumps for both the condenser and chamber. When there are problems with the systems in the lyophilizer, contamination could migrate from the condenser back to the chamber. It is recognized that the condenser is not able to be sterilized in many of the older units, and this represents a major problem, particularly in those cycles in which there is some equipment and/or operator failure.

As referenced above, leakage during a lyophilization cycle can occur, and the door seal or gasket presents an avenue of entry for contaminants. For example, in an inspection, it was noted that during steam sterilization of a lyophilizer, steam was leaking from the unit. If steam could leak from a unit during sterilization, air could possibly enter the chamber during lyophilization.

Some of the newer lyophilizers have double doors - one for loading and the other for unloading. The typical single door lyophilizer opens in the clean area only, and contamination between loads would be minimal. This clean area, previously discussed, represents a critical processing area for a product made by aseptic processing. In most units, only the piston raising/lowering shelves is the source of contamination. For a double door system unloading the lyophilizer in a non-sterile environment, other problems may occur. The non-sterile environment presents a direct avenue of contamination of the chamber when unloading, and door controls similar to double door sterilizers should be in place.

Obviously, the lyophilizer chamber is to be sterilized between batches because of the direct means of contamination. A problem which may be significant is that of leakage through the door seal. For the single door unit, leakage prior to stoppering around the door seal is not a major problem from a sterility concern, because single door units only open into sterile areas. However, leakage from a door gasket or seal from a non-sterile area would present a significant microbiological problem. In order to minimize the potential for contamination, it is recommended that the lyophilizers be unloaded in a clean room area to minimize contamination. For example, in an inspection of a new manufacturing facility, it was noted that the unloading area for double door units was a clean room, with the condenser located below the chamber on a lower level.

After steam sterilization, there is often some condensate remaining on the floor of the chamber. Some manufacturers remove this condensate through the drain line while the chamber is still pressurized after sterilization. Unfortunately, some manufacturers have allowed the chamber to come to and remain at atmospheric pressure with the drain line open. Thus, non-sterile air could contaminate the chamber through the drain line. Some manufacturers have attempted to dry the chamber by blowing sterile nitrogen gas through the chamber at a pressure above atmospheric pressure.

In an inspection of a biopharmaceutical drug product, a Pseudomonas problem probably attributed to condensate after sterilization was noted. On a routine surface sample taken from a chamber shelf after sterilization and processing, a high count of Pseudomonas sp. was obtained. After sterilization and cooling when the chamber door was opened, condensate routinely spilled onto the floor from the door. A surface sample taken from the floor below the door also revealed Pseudomonas sp. contamination. Since the company believed the condensate remained in the chamber after sterilization, they repiped the chamber drain and added a line to a water seal vacuum pump.

FINISHED PRODUCT TESTING FOR

LYOPHILIZED PRODUCTS

There are several aspects of finished product testing which are of concern to the lyophilized dosage form. These include dose uniformity testing, moisture and stability testing, and sterility testing.

(a) Dose Uniformity

The USP includes two types of dose uniformity testing: content uniformity and weight variation. It states that weight variation may be applied to solids, with or without added substances, that have been prepared from true solutions and freeze-dried in final containers. However, when other excipients or other additives are present, weight variation may be applied, provided there is correlation with the sample weight and potency results. For example, in the determination of potency, it is sometimes common to reconstitute and assay the entire contents of a vial without knowing the weight of the sample. Performing the assay in this manner will provide information on the label claim of a product, but without knowing the sample weight will provide no information about dose uniformity. One should correlate the potency result obtained form the assay with the weight of the sample tested.

(b) Stability Testing

An obvious concern with the lyophilized product is the amount of moisture present in vials. The manufacturer's data for the establishment of moisture specifications for both product release and stability should be reviewed. As with other dosage forms, the expiration date and moisture limit should be established based on worst case data. That is, a manufacturer should have data that demonstrates adequate stability at the moisture specification.

As with immediate release potency testing, stability testing should be performed on vials with a known

weight of sample. For example, testing a vial (sample) which had a higher fill weight (volume) than the average fill volume of the batch would provide a higher potency results and not represent the potency of the batch. Also, the expiration date and stability should be based on those batches with the higher moisture content. Such data should also be considered in the establishment of a moisture specification.

For products showing a loss of potency due to aging, there are generally two potency specifications. There is a higher limit for the dosage form at the time of release. This limit is generally higher than the official USP or filed specification which is official throughout the entire expiration date period of the dosage form. The USP points out that compendial standards apply at any time in the life of the article.

Stability testing should also include provisions for the assay of aged samples and subsequent reconstitution of these aged samples for the maximum amount of time specified in the labeling. On some occasions, manufacturers have established expiration dates without performing label claim reconstitution potency assays at the various test intervals and particularly the expiration date test interval. Additionally, this stability testing of reconstituted solutions should include the most concentrated and the least concentrated reconstituted solutions. The most concentrated reconstituted solution will usually exhibit degradation at a faster rate than less concentrated solutions.

(c) Sterility Testing

With respect to sterility testing of lyophilized products, there is concern with the solution used to reconstitute the lyophilized product. Although products may be labeled for reconstitution with Bacteriostatic Water For Injection, Sterile Water For Injection (WFI) should be used to reconstitute products. Because of the potential toxicities associated with Bacteriostatic Water For Injection, many hospitals only utilize WFI. Bacteriostatic Water For Injection may kill some of the vegetative cells if present as contaminants, and thus mask the true level of contamination in the dosage form.

As with other sterile products, sterility test results which show contamination on the initial test should be identified and reviewed.

FINISHED PRODUCT INSPECTION - MELTBACK

The USP points out that it is good pharmaceutical practice to perform 100% inspection of parenteral products. This includes sterile lyophilized powders. Critical aspects would include the presence of correct volume of cake and the cake appearance. With regard to cake appearance, one of the major concerns is meltback.

Meltback is a form of cake collapse and is caused by the change from the solid to liquid state. That is, there is incomplete sublimation (change from the solid to vapor state) in the vial. Associated with this problem is a change in the physical form of the drug substance and/or a pocket of moisture. These may result in greater instability and increased product degradation.

Another problem may be poor solubility. Increased time for reconstitution at the user stage may result in partial loss of potency if the drug is not completely dissolved, since it is common to use in-line filters during administration to the patient.

Manufacturers should be aware of the stability of lyophilized products which exhibit partial or complete meltback. Literature shows that for some products, such as the cephalosporins, that the crystalline form is more stable than the amorphous form of lyophilized product. The amorphous form may exist in the "meltback" portion of the cake where there is incomplete sublimation.

GLOSSARY

ATMOSPHERE, THE EARTH

The envelope of gases surrounding the earth, exerting under gravity a pressure at the earth's surface, which includes by volume 78% nitrogen, 21% oxygen, small quantities of hydrogen, carbon dioxide, noble gases, water vapor, pollutants and dust.

ATMOSPHERIC PRESSURE

The pressure exerted at the earth's surface by the atmosphere. For reference purposes a standard atmosphere is defined as 760 torr or millimeters of mercury, or 760,000 microns.

BACKSTREAMING

A process that occurs at low chamber pressures where hydrocarbon vapors from the vacuum system can enter the product chamber.

BLANK-OFF PRESSURE

This is the ultimate pressure the pump or system can attain.

BLOWER (see Mechanical Booster Pump)

This pump is positioned between the mechanical pump and the chamber. It operates by means of two lobes turning at a high rate of speed. It is used to reduce the chamber pressure to less than 20 microns.

BREAKING VACUUM

Admitting air or a selected gas to an evacuated chamber, while isolated from a vacuum pump, to raise the pressure towards, or up to, atmospheric.

CIRCULATION PUMP

A pump for conveying the heat transfer fluid.

CONDENSER (Cold trap)

In terms of the lyophilization process, this is the vessel that collects the moisture on plates and holds it in the frozen state. Protects the vacuum pump from water vapor contaminating the vacuum pump oil.

CONDENSER/RECEIVER

In terms of refrigeration, this unit condenses (changes) the hot refrigerant gas into a liquid and stores it under pressure to be reused by the system.

COOLING

The lowering of the temperature in any part of the temperature scale.

CONAX CONNECTION

A device to pass thermocouple wires through and maintain a vacuum tight vessel.

CONTAMINATION

ln the vacuum system, the introduction of water vapor into the oil in the vacuum pump, which then causes the pump to lose its ability to attain its ultimate pressure.

DEFROSTING

The removal of ice from a condenser by melting or mechanical means.

DEGREE OF CRYSTALLIZATION

The ratio of the energy released during the freezing of a solution to that of an equal volume of water.

DEGREE OF SUPERCOOLING

The number of degrees below the equilibrium freezing temperature where ice first starts to form.

DESICCANT

A drying agent.

DRY

Free from liquid, and/or moisture.

DRYING

The removal of moisture and other liquids by evaporation.

EQUILIBRIUM FREEZING TEMPERATURES

The temperature where ice will form in the absence of supercooling.

EUTECTIC TEMPERATURE

A point of a phase diagram where all phases are present and the temperature and composition of the liquid phase cannot be altered without one of the phases disappearing.

EXPANSION TANK

This tank is located in the circulation system and is used as a holding and expansion tank for the transfer liquid.

FILTER OR FILTER/DRIER

There are two systems that have their systems filtered or filter/dried. They are the circulation and refrigeration systems. In the newer dryers this filter or filter/dryer is the same, and can be replaced with a new core.

FREE WATER

The free water in a product is that water that is absorbed on the surfaces of the product and must be

removed to limit further biological and chemical reactions.

FREEZING

This is the absence of heat. A controlled change of the product temperature as a function of time, during the freezing process, so as to ensure a completely frozen form.

GAS BALLAST

Used in the vacuum system on the vacuum pump to decontaminate small amounts of moisture in the vacuum pump oil.

GAS BLEED (Vacuum control)

To control the pressure in the chamber during the cycle to help the drying process. In freeze-drying the purpose is to improve heat-transfer to the product.

HEAT EXCHANGER

This exchanger is located in the circulation and refrigeration systems and transfers the heat from the circulation system to the refrigeration system.

HEAT TRANSFER FLUID

A liquid of suitable vapor pressure and viscosity range for transferring heat to or from a component, for example, a shelf or condenser in a freeze-dryer. The choice of such a fluid may depend on safety considerations. Diathermic fluid.

HOT GAS BYPASS

This is a refrigeration system. To control the suction pressure of the BIG FOUR (20-30 Hp) compressors during the refrigeration operation.

HOT GAS DEFROST

This is a refrigeration system. To defrost the condenser plates after the lyophilization cycle is complete.

ICE

The solid, crystalline form of water.

INERT GAS

Any gas of a group including helium, radon and nitrogen, formerly considered chemically inactive.

INTERSTAGE

In a two stage compressor system, this is the cross over piping on top of the compressor that connects the low side to the high side. One could also think of it as low side, intermediate, and high side.

INTERSTAGE PRESSURE REGULATING VALVE

This valve controls the interstage pressure from exceeding 80 - 90 PSI. This valve opens to suction as the interstage pressure rises above 80 - 90 PSI.

LEXSOL

A heat transfer fluid (high grade kerosene).

LIQUID SUB-COOLER HEAT EXCHANGER (see Sub-cooled Liquid)

The liquid refrigerant leaving the condenser/receiver at cooling water temperature is sub-cooled to a temperature of +15oF (-10oC) to -15oF (-25oC).

LYOPHILIZATION

A process in which the product is first frozen and then, while still in the frozen state, the major portion of the water and solvent system is reduced by sublimation and desorption so as to limit biological and chemical reactions at the designated storage temperature,

MAIN VACUUM VALVE (see Vapor Valve)

This valve is between the chamber and external condenser to isolate the two vessels after the process is finished. This is the valve that protects the finished product.

MATRIX

A matrix, in terms of the lyophilization process, is a system of ice crystals and solids that is distributed throughout the product.

MECHANICAL BOOSTER PUMP (see Blower)

A roots pump with a high displacement for its size but a low compression ratio. When backed by an oil-seal rotary pump the combination is an economical alternative to a two-stage oil-sealed rotary pump, with the advantage of obtaining a high vacuum.

MECHANICAL VACUUM PUMP

The mechanical pumping system that lowers the pressure in the chamber to below atmospheric pressure so that sublimation can occur.

MELTING TEMPERATURE (Melt-back)

That temperature where mobile water first becomes evident in a frozen system.

MICRON (see Torr)

A unit of pressure used in the lyophilization process. One micron = one Mtorr or 25,400 microns = 1" Hg., or 760,000 microns = one atmosphere.

NONCONDENSABLES

A mixture of gases such as nitrogen, hydrogen, chlorine, and hydrocarbons. They may be drawn into the system through leaks when part of the system is under a vacuum. Their presence reduces the operating efficiency of the system by increasing the condensing pressure.

NUCLEATION

The formation of ice crystals on foreign surfaces or as a result of the growth of water clusters.

OIL-MIST FILTER

In vacuum terminology a filter attached to the discharge (exhaust) of an oil-sealed rotary pump to eliminate most of the "smoke" of suspended fine droplets of oil which would be discharged into the environment.

OIL SEALED ROTARY PUMP

A standard type of mechanical vacuum pump used in freeze-drying with a high compression ratio but having a relatively low displacement (speed) for its size. A two-stage pump is effectively two such pumps in series and can obtain an ultimate vacuum.

OIL SEPARATOR

Separates the oil from the compressor discharge gas and returns the oil through the oil float trap and piping to the compressor crankcase.

REAL LEAK

A real leak is a source of atmospheric gases resulting from a penetration through the chamber.

RECONSTITUTE

The dissolving of the dried product into a solvent or diluent.

RELIEF VALVE

Used for safety purposes to prevent damage in case excessive pressure is encountered.

ROTARY VANE PUMP

A mechanical pumping system with sliding vanes as the mechanical seal. Can be single or two stages.

SHELF COMPRESSOR (Controlling Compressor)

Used for controlling the shelf temperature, either cooling or from overheating.

SELF LIQUID HEAT EXCHANGER

The transfer of heat from the shelf fluid to the refrigeration system through tubes in the exchanger causing compressor suction gas to warm.

SHELVES

In terms of the lyophilization process, they are a form of heat exchanger, within the chamber, that have a serpentine liquid flow through them, entering one side and flowing to the other side. They are located in the circulation system.

SINGLE STAGE COMPRESSOR

This is a normal type compressor used in refrigeration. In the lyophilization process it is used to control the shelf temperature, both for cooling and keeping the shelf temperature from overheating using a temperature controller.

SILICONE OIL

A heat transfer fluid.

STERILIZATION

The use of steam and pressure to kill any bacteria that may be able to contaminate that environment or vessel.

SUBLIMATION

The conversion of a material from a solid phase directly to a vapor phase, without passing through the liquid phase. This is referred to as the primary drying stage.

SUB-COOLED LIQUID (See Liquid Sub-cooler Heat Exchanger)

The liquid refrigerant is cooled through an exchanger so that it increases the refrigerating effect as well as reduces the volume of gas flashed from the liquid refrigerant in passage through the expansion valve.

SUCTION LINE ACCUMULATOR

To provide adequate refrigerant liquid slug protection (droplets of liquid refrigerant) from returning to the compressor, and causing damage to the compressor.

TCE

Trichloroethylene - A heat transfer fluid.

TEMPERATURE

The degree of hotness or coldness of a body.

THERMOCOUPLE

A metal-to-metal contact between two dissimilar metals that produces a small voltage across the free ends of the wire.

THERMOSTATIC EXPANSION VALVE

An automatic variable device controlling the flow of liquid refrigerant.

TORR (See Micron)

A unit of measure equivalent to the amount of pressure in 1000 microns.

TWO STAGE COMPRESSOR (see Interstage)

This is a specially built compressor. Its function is to be able to attain low temperatures by being able to operate at low pressures. It is two compressors built into one. A low stage connected internally and a high stage connected externally with piping, called interstage.

UNLOADING VALVE

This valve connects the interstage with suction to equalize both pressures during pump-down.

VACUUM

Strictly speaking, a space in which the total pressure is less than atmospheric.

VACUUM CONTROL (Gas Bleed)

To assist in the rate of sublimation, by controlling the pressure in the lyophilizer.

VACUUM PUMP

A mechanical way of reducing the pressure in a vessel below atmospheric pressure to where sublimation can occur. There are three types of pumps, rotary vane, rotary piston and mechanical booster.

VAPOR BAFFLE

A target shaped object placed in the condenser to direct vapor flow and to promote an even distribution of condensate.

VACUUM VALVES

The vacuum valves used are of a ball or disk type that can seal without leaking. The balI types are used for services to the chamber and condenser. They are also used for drains and isolation applications. The disk types are used in the vacuum line system and are connected to the vacuum pump, chamber and condenser.

VAPOR VALVE (See Main Vacuum Valve)

The vacuum valve between the chamber and external condenser. When this valve is closed the chamber is isolated from the external condenser. Also known as the main vapor valve.

VIAL

A small glass bottle with a flat bottom, short neck and flat flange designed for stoppering.

VIRTUAL LEAK

In the vacuum system a virtual leak is the passage of gas into the chamber from a source that is located internally in the chamber.