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Verification of Compounding Accuracy and Sterility

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* The author acknowledges Philip J. Schneider who authored this chapter in the previous edition.

Introduction

A majority of compounded sterile preparations (CSPs) prepared in healthcare institutions are low-risk or medium-risk level prepared via manual manipulation of sterile components within closed transfer systems (e.g. vials, bags, and syringe/needles). The USP Chapter <797> section entitled, “Verification of Compounding Accuracy and Sterility” focuses on the critical activities associated with preparing CSPs from non-sterile ingredients and/or non-sterile devices.¹ This type of compounding is considered high-risk level compounding and as such requires a greater degree of expertise and understanding from compounding personnel in achieving and maintaining the sterility, accuracy, and purity of finished CSPs. Compounding personnel engaged in high-risk compounding should consult additional expert references on the topics of sterility testing and bacterial endotoxin testing, which must be performed in accordance with USP Chapter <71> and USP Chapter <85>.^{2,3}

Principles of Sterility

Compounders of high-risk CSPs must understand the principles of sterility by understanding the relationship between the Probability of a Non-Sterile Unit (PSNU) and rates of contamination. The definition of sterility is the absence of viable microorganisms. Sterility is an absolute concept and a CSP will either be sterile or it will be contaminated.

Rates of contamination involve the number of compounded preparations that are nonsterile within a batch after preparation. PSNU is defined as the probability of an item being nonsterile after it has been exposed to a validated sterilization process (e.g. validated filtration, steam, dry heat, ionizing radiation, ethylene oxide). Terminally sterilized commercial sterile pharmaceutical products typically have a PSNU of 10^{-6} (the probability of one in a million compounded and sterilized items being nonsterile).

Pharmacy-prepared CSPs compounded via aseptic compounding procedures (no final filtration or terminal sterilization) can ONLY claim a PSNU of no more than 10^{-3} (the probability of one in a thousand items being nonsterile), assuming that a robust compounding procedure has been conducted. A PSNU of 10^{-3} will be highly dependent on working in a properly designed and operational controlled environments (ISO Class 5,

7 and 8 areas), properly trained and competent compounding personnel, excellent work practices designed to minimize the risk of touch contamination (critical site protection and working properly within the direct compounding area) and mitigating the negative affects from inbound and outbound compounding ingredients and personnel. One of the hardest things to control is the people involved in the compounding process. The most common reason for contamination of a product is touch ⁴⁻⁹.

The importance of verifying compounding processes is critical to support any assigned beyond-use dating which is best done through aseptic technique media fills. It must be assumed that at least 1 in 1,000 CSPs prepared aseptically will be nonsterile. This is why all other supportive functions and activities (e.g. employee training, competency and proficiency, facility design, cleaning, and garbing and gloving) are so vitally important to the sterility of the final CSP. These factors are covered in other chapters.

Quality of Bulk Ingredients

When starting the process of verifying the accuracy and sterility of a compounded sterile preparation, the quality of the ingredients must be verified. Discussion on Certificates of Analysis and the other requirements associated with bulk pharmaceutical ingredients, also known as active pharmaceutical ingredients (APIs) is covered in the Chapters 2 and 24. Some bulk containers of API will not have an expiration date on their containers. In such instances, these containers should be verified against their Certificates of Analysis, dated upon receipt into inventory and dated, and initialed when opened and assigned a one-year expiration date.¹⁰ Although these ingredients may be chemically stable indefinitely, they may gain or lose moisture (water of hydration) during storage and use. It is important to have an understanding of the nature of ingredients being used and their chemical properties so if an ingredient is hygroscopic or loses moisture over time; it can be compensated for during the storage period only by chemical analysis according to chemical monograph standards. Additional testing may be required in accordance with USP Chapter <731> - Loss on Drying, when weighing out the ingredient.

Methods of Sterilization

Sterilization is the process that is intended to kill or remove all types of microorganisms. There are two principal terminal sterilization methods:

1. Physical (filtration, saturated steam or dry heat)
2. Chemical (ethylene oxide gas or chemical liquids)

Factors, which determine the method to be used, are the types of microorganisms involved, the nature of the article to be sterilized and the time available for sterilization.

Filtration is the most common sterilization method used during extemporaneous compounding of high-risk level CSPs performed by pharmacists and technicians. Regardless of the method used, the sterilization cycle must be evaluated to ensure that it was adequate.¹

Sterilization by filtration

Different types of filters may be used when compounding sterile preparations. Any filter used to sterilize pharmacy-prepared sterile preparations must have a nominal pore size of 0.2 or 0.22 micron. 0.22 micron filters will remove microorganisms, particles, precipitates, and undissolved powders larger than 0.22 micron.¹¹

The filter must be a commercially-available sterilizing grade filter approved for human-use. Not all filters used in pharmacies today comply with this requirement. Sterilizing grade filters have been tested and certified by the manufacturer to retain at least 10^7 microorganisms of a strain of *Brevundimonas (Pseudomonas) diminutia* on each square centimeter of upstream filter surface area under conditions similar to those in which the CSP will be sterilized under normal use.

A pharmacist must ensure that the filter being used has a certificate of quality provided by the filter vendor that will prove the filter has been tested. In addition to microbiological retention information, the vendor should also have testing information for membrane and housing integrity, nonpyrogenicity, and extractables.¹²

Filter selection

Filters work by different mechanisms. They include sieving, adsorption and entrapment.¹³ Filters work through a combination of multiple mechanisms. The effectiveness of the filtration process is influenced by the microbial burden of the solution to be filtered.¹⁴

When deciding which filter to use to sterilize a solution, considerable care must be taken to choose the correct filter.

The pharmacist should select the appropriate filter size, based on the volume of solution to be filtered and the particulate load so that a single filter can be used to process an entire batch. Filters should not have to be replaced during the filtration process. The selection of a filter should be based on the characteristics of the filter and of the solution. Filters and their housing apparatus must be physically and chemically compatible with the solution and be capable of withstanding processing temperatures and pressures. When filters are selected, four characteristics should be considered:

Pore size

Compatibility

Fluid volume

Particulate load

Reference 11 offers a more in-depth discussion of filter selection.

Pore size: Pore size determines the size of the particles retained within the membrane.

Compatibility: Membrane filters are compatible with most aqueous solutions but interactions between the filter media and solution can occur. The most common interaction is caused by sorption or leaching. Sorption is the binding of drug or other formulation component to the filter. Large molecular weight drugs, peptides, proteins or emulsions may be adversely affected by filtration. The passage through a small pore size filter may cause shear stress and alter the three dimensional structure of drug or solution, rendering them pharmacological inactive.¹² Leaching is the extracting of substances used in the manufacture of the filter into the filtered solution. Substances, such as surfactants are added to the filter make it hydrophilic, and can leach into filtered solution. These components should not be present in the CSP. However, most membrane filters are designed to be compatible with most pharmaceutical solutions. All filter manufacturers have compatibility data on their membrane types and should be consulted to ensure that the drug or solution being filtered is chemically and physically compatible with the filter housing and filter membrane material at the pressure and temperature conditions that are used.

Fluid volume. To provide a practical flow rate of a solution, a filter with the appropriate surface area must be used (i.e., the larger the volume of solution, the greater the amount of filter membrane surface area required). In the pharmacy, the volume of solution might range from a few milliliters to a few liters; a disk filter of 25-mm or 33-mm diameter often is suitable.

Most 25-33 mm syringe disk filters used in pharmacy compounding applications to sterilize solutions have a maximum process volume of no more than 100 mLs of solution.¹¹ If larger volumes of solution (>100mL) have to be filtered, a cartridge filter with more surface area may be required based on the volume of solution and desired flow rate.¹³

Particulate load. Most pharmaceutical solutions are compounded under controlled conditions where the environmental air cleanliness is controlled, and the load of particles in solution is low. Some preparations may have a large load (e.g., an impure or insoluble drug). Choose the appropriate size and configuration of filtration device that will accommodate the volume being filtered to permit complete filtration without clogging of the membrane. If the solution that is being filtering has a heavy particulate load, a 5-micron or 1.2 micron pre-filter should be used upstream of the 0.2-micron filter to decrease the particulate load on the 0.2-micron filter.

Some preliminary experimentation may be needed to determine the rate of filter clogging. If the particulate load is relatively low (e.g., glass particles in a sterile solution after an ampul is opened), a 5-micron membrane filter may be used.¹³

Hydrophobic and hydrophilic filters

Hydrophilic membranes wet spontaneously with water. They are used for filtration of aqueous solutions and aqueous solutions containing water miscible solvents. Hydrophobic filters do not wet spontaneously with water. They are used for filtering gases and solvents.

General Filter Cautions

The following is a summary of the general cautions when using filters: ¹¹

- A filter is single use devices. Do not resterilize or reuse

- Use caution with syringes smaller than 10 mL since the pressure generated by these syringes can exceed the bubble-point of the filter (>75 psi). Ideally, a 60 mL syringe should be used to minimize the pressure generated by the syringe.
- Do not use the same filter to filter solution in both directions of the filter
- Use caution when filtering solution containing 5mg or less of API unless binding studies have been performed.

Filter integrity

One of the critical methods of verifying the performance of a sterilizing grade filter is to perform a bubble-point or filter integrity test. USP Chapter <797> requires that all membrane filter assemblies used to sterilize a compounded sterile preparation be subjected to the manufacturer's recommended integrity test. This integrity test or bubble point is a simple, nondestructive check of the integrity of the filtration assembly, including the filter membrane.¹

The principle behind the test is based on the fact that liquid is held in the capillary structure of the membrane by surface tension. The minimum pressure required to force the liquid out of the capillary space is a measure of the largest pore in the membrane, and is reported as pounds per square inch (psi). Each filter has a manufacturer-specified bubble point. Certain drug preparations may lower the bubble point of the filter, resulting in air passing through it at a lower pressure than expected. If the test is questionable, the preparation should be resterilized. Chapter 13 Figure -1 describes a sample procedure for filter integrity testing of small disk filters.

Principles of Sterilization

Since steam sterilization is considered an advanced compounding procedure, it is strongly recommended personnel consult with additional compendial and expert references like USP Chapter <1211> Sterilization and Sterility Assurance of Compendial Articles¹⁴ and the Parenteral Drug Associations Technical Report No. 1, Revised 2007.¹⁵ Steam or moist heat sterilization involves the use of an autoclave and dry heat sterilization involves the use of an oven. Either process requires the compounder to use methods to minimize sources of contamination

- Personnel garbing and hand hygiene
- Cleaning and disinfection
- Controlled environments

Sterility Assurance Level (SAL) and Probability of a nonsterile unit (PNSU)

Sterility Assurance Level (SAL) is defined as the “probability of a single microorganism occurring on an item after sterilization.”¹⁶ In 1984, an FDA publication stated “the desired sterility assurance level (SAL), or probability of a unit being nonsterile after exposure to a valid sterilization process, varies according to the intent use of the device.”¹⁷ A simple definition might be the probability that a given sterilization process failed to destroy all of the microorganisms.¹⁸ For preparations that may be damaged by excessive heat exposure, the development of sterilization cycles depends on knowledge of the microbial burden of the preparation.

There are only two possible outcomes of a sterilization cycle, either the CSP sterile or it is not sterile. The sum of the probabilities for both outcomes must equal 100%.¹⁹ The probability of a nonsterile unit (PNSU) is expressed as a probability. With heat-stable items, the approach often is to “overkill” to exceed the critical time necessary to achieve the 10^{-12} microbial survivor probability.

Sterilization by Steam

Steam sterilization (a.k.a. autoclaving, or moist heat sterilization) depends on the use of steam above 100°C. This form of terminal sterilization is the preferred method to sterilize aqueous solutions and suspensions that have been verified to maintain their chemical and physical stability under this method.

There are three expressions that need to be explained. The first is the D (decimal reduction time) value. It is a logarithmic expression and is the amount of time at a certain temperature required to kill 90% of the organisms that may be present. It is expressed as the time in minutes required to reduce the microbial population of a specific solution by 90% or one log cycle at a specific temperature. For example, if the population of 100 microorganisms in a solution is reduced by 1 D, the number of microorganisms has been decreased by 90% or 1 decimal place or one log. Ten (10) organisms will have survived

the exposure. The second expression is the Z-Value. It is defined as the temperature coefficient of microbial destruction or the difference in the number of degrees of temperature to change the D-value one log unit in time. This would cause a 10-fold variation of the D value. Chapter 13 Figure 2 illustrates both of these concepts. The last expression is the F-value. It is a calculated number that takes the entire temperature profile of the sterilization process into account and a numerical equivalency of the lethality of the treatment at 121° C for “x” number of minutes.²⁰

Sterilization cycles should be validated to ensure that the survival of the most resistant microorganisms is no greater than 10^{-6} under specified operating conditions and parameters (e.g., sterilization time and temperature, size and nature of load, and chamber loading configuration). The validation and monitoring of heat sterilization should be documented along with specific critical parameters, such as necessary temperatures, pressures, and use of commercially available biologic indicators (BIs). Monitoring data from each cycle must be recorded to ensure that the processes are performed properly and that all critical parameters are within specified limits during compounding.¹⁴

Steam sterilization must be reserved for items that can be penetrated by water vapor. Therefore, dry sealed containers, oils, and waxes may not be suitable for sterilization via autoclaving. Steam sterilization has the advantage of rapid penetration of wrapped materials with the destruction of all viruses and bacteria, including the most resistant spores. The sterilization of different supplies is more readily controlled than in other types of sterilizers. However oils, grease and powdered substances cannot be sterilized by this method. The steam autoclave must be maintained in good repair and operated correctly in order to perform to specifications. Sterilization failure can occur when machines are not regularly serviced.

The effectiveness of steam sterilization must be verified using appropriate BIs of *Geobacillus stearothermophilus* (see Biological Indicators <1035>) and other confirmation methods such as temperature-sensing devices (see Sterilization and Sterility Assurance of Compendial Articles <1211> and Sterility Tests <71>).¹

Sterilization by Gas

Ethylene oxide gas is effective against all types of microorganisms. The biocidal action of this gas is considered to be alkylation of nucleic acids. It is non-corrosive and safe for most plastic and polyethylene materials. However, it is not applicable to liquids or to articles in impervious packaging material. It cannot be used to sterilize animal diets due to the potential toxic effects of this gas. It can also be a toxic hazard for animals receiving prosthetic implants, which have been sterilized by this gas.

Ethylene oxide gas is a potential carcinogen and mutagen and represents a potential occupational health hazard for personnel operating sterilizers. Operation of gas sterilizers and aerators should be in strict conformance with manufacturers' recommendations and institutional policies. Personnel exposure should be minimized by appropriate ventilation of exhaust gas. A regular monitoring program for personnel should be in place.

The use of chemical sterilants is outside the scope of ordinary pharmacy compounding practice and needs to be performed by qualified experts.

Sterilization and Depyrogenation by Dry Heat

This method should be used only for materials that might be damaged by moist heat or that are impenetrable by moist heat (e.g., powders, petroleum products, sharp instruments). The advantages for dry heat include the following: it is nontoxic and does not harm the environment; a dry heat cabinet is easy to install and has relatively low operating costs; it penetrates materials; and it is noncorrosive for metal and sharp instruments. The disadvantages for dry heat are that the slow rate of heat penetration and microbial killing makes this a time-consuming method and the high temperatures are not suitable for most materials. The most common time-temperature relationships for sterilization with hot air sterilizers are 170°C (338°F) for 60 minutes, 160°C (320°F) for 120 minutes, and 150°C (302°F) for 150 minutes. *B. subtilis* spores should be used to monitor the sterilization process for dry heat because they are more resistant to dry heat than are *G. stearothermophilus* spores. The primary lethal process is considered to be oxidation of cell constituents. There are two types of dry-heat sterilizers: the static-air type and the forced-air type. The static-air type is referred to as the oven-type sterilizer as heating coils in the bottom of the unit cause the hot air to rise inside the chamber via

gravity convection. This type of dry-heat sterilizer is much slower in heating, requires a longer time to reach sterilizing temperature, and is less uniform in temperature control throughout the chamber than is the forced-air type. The forced-air or mechanical convection sterilizer is equipped with a motor-driven blower that circulates heated air throughout the chamber at a high velocity, permitting a more rapid transfer of energy from the air to the contents.²¹

Dry-heat depyrogenation continues to be the method of choice for the sterilization and depyrogenation of glassware. Any depyrogenation process must be validated to yield at least a three-log reduction of reference-standard bacterial (*E. Coli*) endotoxin. The resistance of endotoxin to heat is at least an order of magnitude greater than even the most resistant spore, therefore a three-log or more reduction in endotoxin results in a very low probability of nonsterility, far lower in fact than 10^{-6} (SAL $>10^6$).²²

Summary

Great care must be taken when compounding sterile preparations that are considered high-risk level CSPs. It requires a greater degree of expertise and understanding from compounding personnel when working with nonsterile ingredients and/or devices. The sterility, accuracy, and purity of finished CSPs must be achieved and maintained at all times. General guidelines for matching CSPs and components to appropriate sterilization methods include the following:¹

1. CSPs have been ascertained to remain physically and chemically stable when subjected to the selected sterilization method.
2. Glass and metal devices may be covered tightly with aluminum foil, then exposed to dry heat in an oven at a mean temperature of 250° C for 30 minutes to achieve sterility and depyrogenation (see Dry-Heat Sterilization under Sterilization and Sterility Assurance of Compendial Articles <1211> and Bacterial Endotoxins Test <85>). Such items are either used immediately or stored until use in an environment suitable for compounding low-risk level CSPs and medium-risk level CSPs.

3. Personnel ascertain from appropriate information sources that the sterile microporous membrane filter used to sterilize CSP solutions, during either compounding or administration, is chemically and physically compatible with the CSP.

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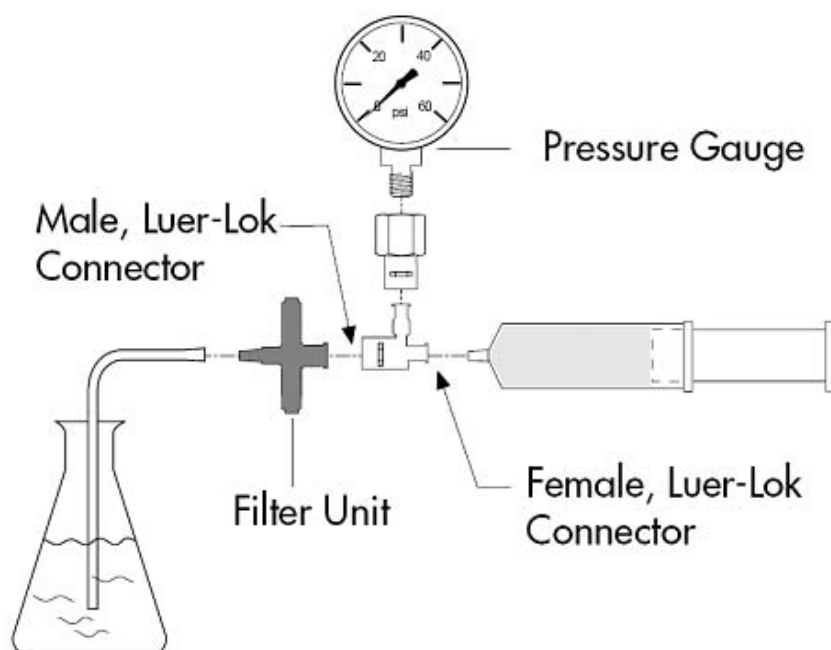
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Filter Integrity Testing

Millex/Sterivex Integrity Tester

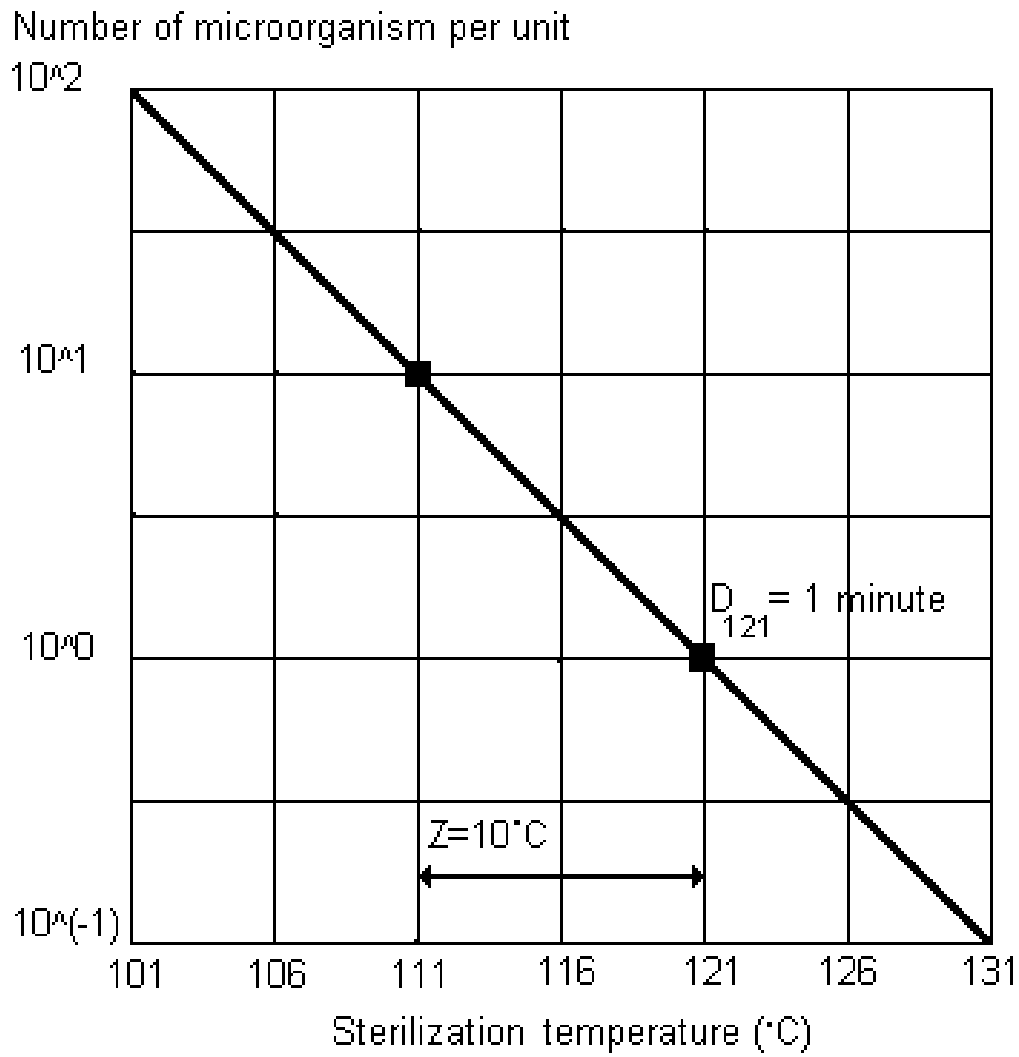


Bubble Point Procedure:

1. Wet the filter with the appropriate fluid, typically water for hydrophilic membranes or an alcohol/water mixture for hydrophobic membranes.
2. Pressurize the system to about 80% of the expected bubble point pressure which is stated in the manufacturer's literature.
3. Slowly increase the pressure until rapid continuous bubbling is observed at the outlet.
4. A bubble point value lower than the specification is an indication of one of the following:
 - fluid with different surface tension than the recommended test fluid
 - integral filter, but wrong pore size
 - high temperature
 - incompletely wetted membrane
 - non-integral membrane or seal

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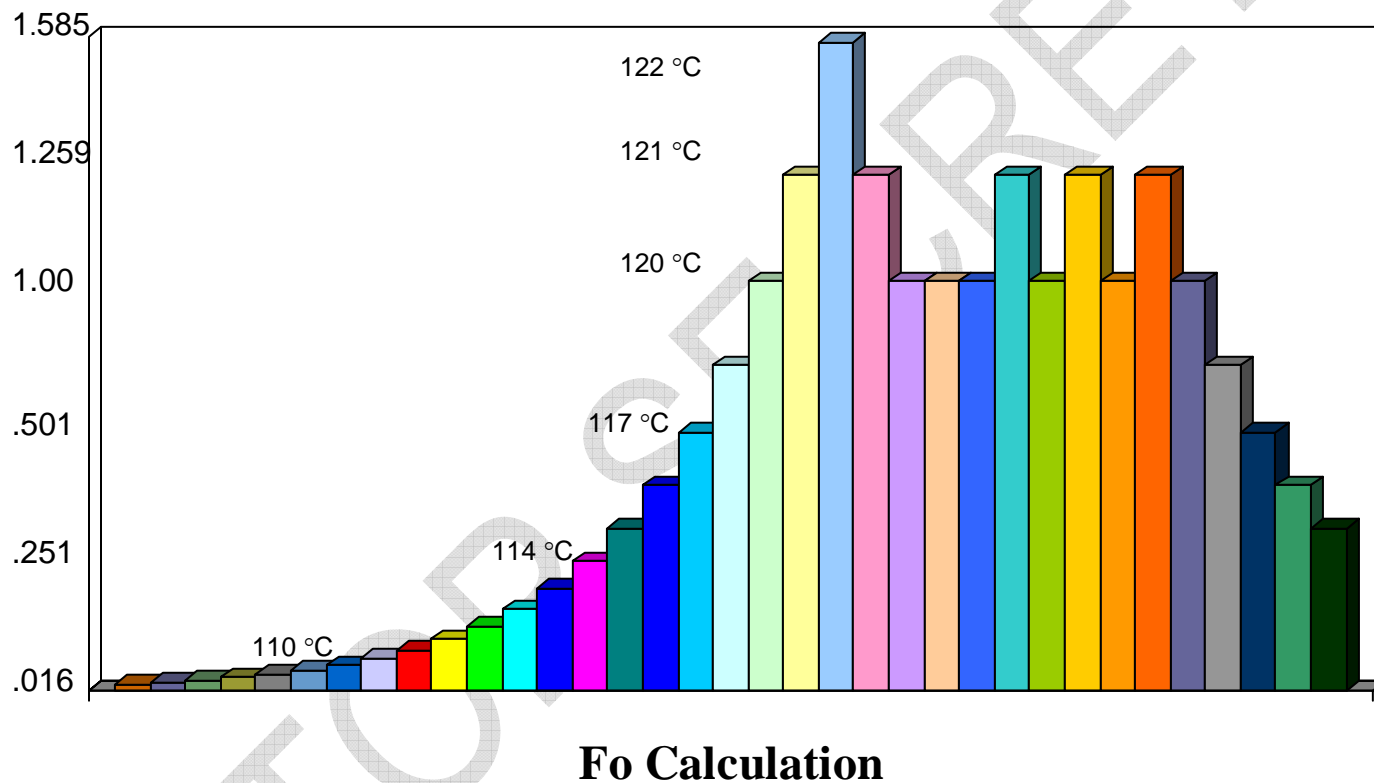
Chapter 13, Figure 2



Chapter 13, Figure 3

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F₀ as a Function of Temperature



*Reference Temperature 120°C
Z - Value = 10 °C