Sterilization

is the process designed to produce a sterile state. The traditional concept of the sterile state is the absolute condition of total destruction or elimination of all living microorganisms.

This concept has given way to the reality that sterile is a term that must be given relative connotation and that the probability of having achieved the absolute can only be predicted on the basis of kinetic projection of microbial death rates. Therefore, sterility in the absolute sense cannot be shown to have been achieved, but rather, can be approached with an increasing probability of success as a sterilization process is improved. With terminal methods of sterilization of a parenteral product, particularly steam under pressure, a probability of no more than one nonsterile unit in a million (10^{-6}) is readily achievable.

Even greater levels of assurance can be achieved with current technology. In this chapter, *sterile* indicates a probable condition of complete freedom from viable microorganisms with the limitations just expressed; these limitations are developed more fully later in the chapter. The term *aseptic* indicates a controlled process or condition in which the level of microbial contamination is reduced to the degree that microorganisms can be excluded from a product during processing. It describes an "apparently" sterile state.

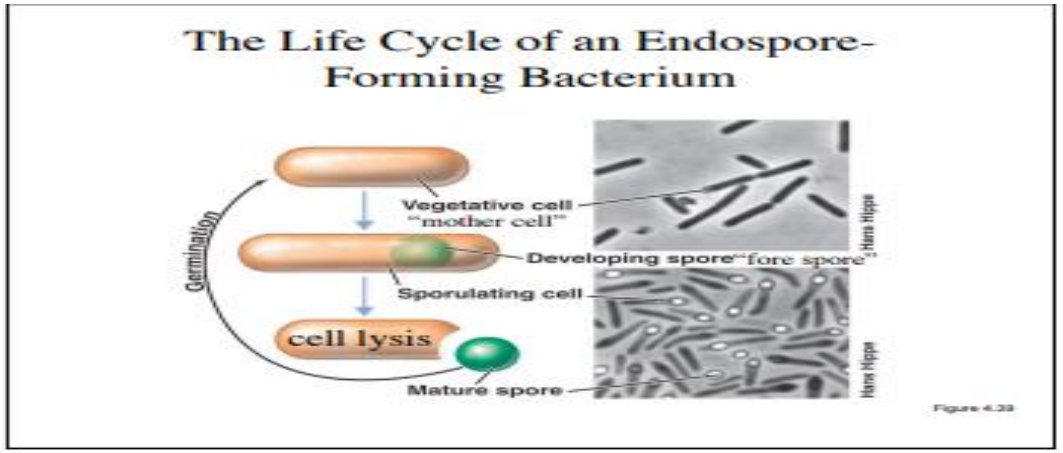
Persons responsible for carrying out sterilization procedures must be acutely aware of the degree of effectiveness as well as the limitations of each sterilization process.

They must also understand that these processes may have a deleterious effect on the material to be sterilized. In the processing of pharmaceuticals, it is often necessary to reach a compromise between the most effective sterilization procedure and one that will not have a significant adverse effect upon the material to be sterilized.

For example, it may be necessary to add an antibacterial agent to a thermally sensitive product to enhance the effectiveness of a low-temperature sterilization process; thereby decomposition is prevented while the combined effect of the antibacterial and the heat provide reasonable assurance that the product will be sterilized.

Microorganisms exhibit varying resistance to sterilization procedures. The degree of resistance varies with the specific organism. In addition, **spores, the form that preserves certain organisms during adverse conditions, are more resistant than vegetative forms of the organism.**

The data given in Table 21-1 illustrate the varying resistance of different spores to moist and dry heat. Therefore, the conditions required for a sterilization process must be planned to be lethal to the most resistant spores of microorganisms normally encountered, with additional treatment designed to provide a margin of safety against a sterilization failure.



file:///C:/Users/User/Downloads/Endosporeformation3.pdf

TABLE 21-1. *Times Required for Lethal Effect on Bacterial Spores by Thermal Exposure*

	Time (min.)					
	Moist Heat			*Dry Heat*		
Organisms	*100°C*	*110°C*	*121°C*	*120°C*	*140°C*	*170°C*
B. anthracis	5–15	—	—	—	180	—
Cl. botulinum	330	90	10	120	60	15
Cl. welchii	5–10	—	—	50	5	7
Cl. tetani	5–15	—	—	—	15	—
Soil bacilli	>1020	120	6	—	—	15

Validation of Sterilization Processes

All sterilization processes (thermal, chemical, radiation, and filtration) are designed to destroy or eliminate microbiologic contaminants present in a product.

The official test for sterility of the product is a destructive test on a selected sample; thus, the task of proving that all units of a product are sterile must involve the employment of probability statistics. The statistics of probability depend on such parameters **as the length or degree of exposure to the sterilant,** **the type and number of microorganisms present**, **the desired level of microbial destruction or elimination**, and the **resistance of the microorganism(s) presented** to the sterilization process.

In recent years, the pharmaceutical industry has intensified its efforts to quantitate the rate and extent of microbial destruction or elimination.
The Food and Drug Administration has stated in its current good manufacturing practice regulations that sterilization procedures must be validated pertaining to (1) the design of the equipment and the process used to produce batch sterilization and
(2) the confirmation with reproducible data of a given probability level of residual microbial contamination upon completion of the sterilization process.

Validation of sterilization processes can be facilitated by using quantitative, theoretically sound principles such **as microbial death kinetic expressions**.

Microbial Death Kinetic Terms

An important term in expressing microbial death kinetics for heat, chemical, and radiation sterilization is the **D *value***.

The D value is the time (for heat or chemical exposure) or the dose (for radiation exposure) required for the microbial population to decline by one decimal point (a 90%, or one logarithmic unit, reduction).

The D value may be estimated graphically, as shown in Figure 21-1, or mathematically, as shown by equation (1):

$$D = \frac{U}{\log N_0 - \log N_u} \qquad (1)$$

where **U** is the exposure time or exposure dose, under specific conditions, $\mathbf{N_0}$ is the initial microbial population (product bioburden) and **Nu** is the microbial population after receiving U time or dose units of sterilant exposure.

For example, after 5 min of product exposure to a temperature of 121°C, the microbial population was reduced from 2 x 10^5 to 6 x 10^3. Then, the D value at 121°C is:

$$D_{121} = \frac{5 \text{ min}}{\log(2 \times 10^5) - \log(6 \times 10^3)} = 3.28 \text{ min}$$

Thus, at 121 °C, the microbial population is decreased by 90% every 3.28 min.

D values have been defined precisely for various microorganisms contained. In certain environments (liquids and solid surfaces) at specific temperatures for heat sterilization, and at direct exposure to cobalt-60 irradiation.

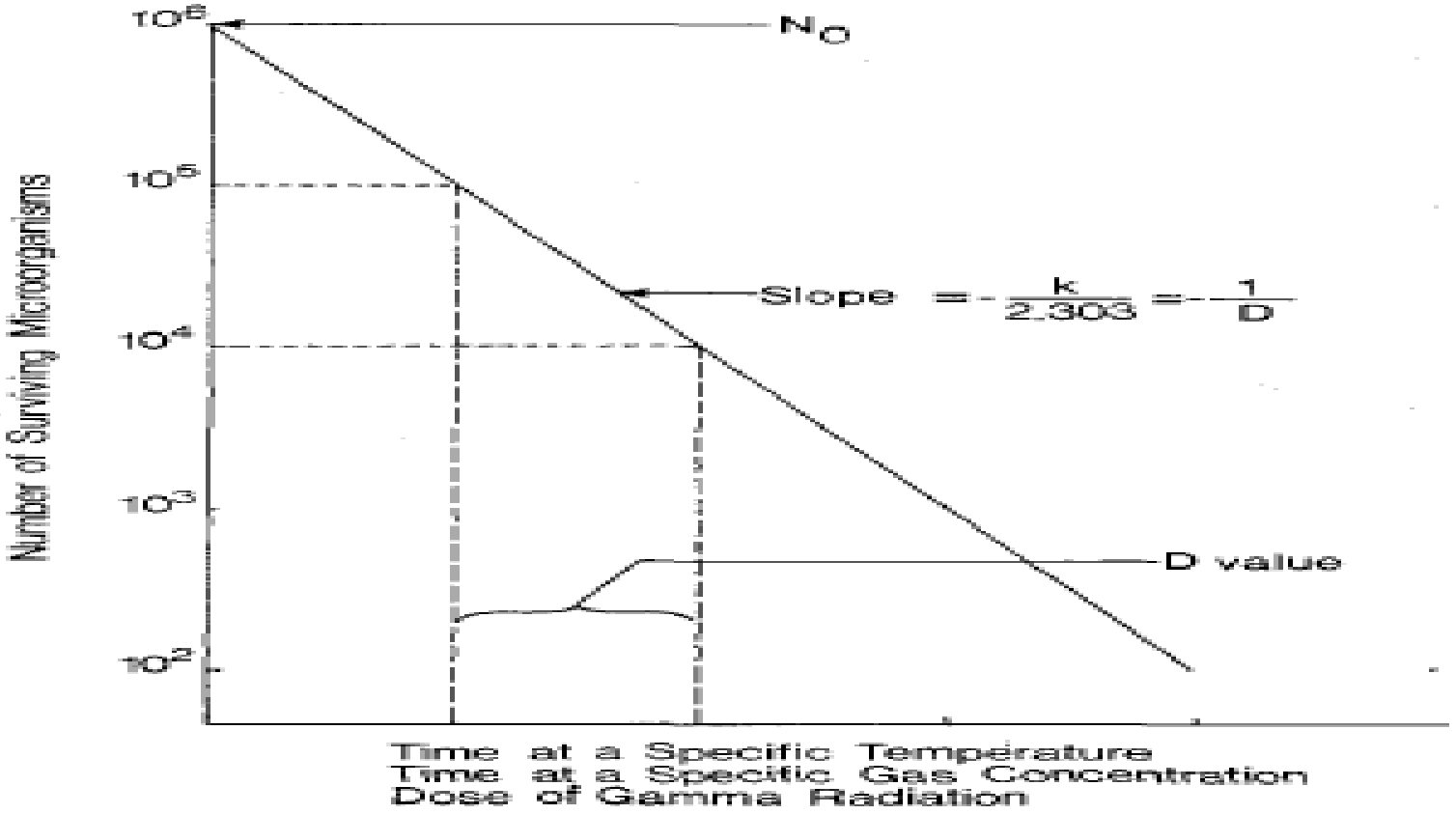


FIG. 21-1. *Graphic representation of the semilogarithmic microbial death rate.*

D values can not be defined precisely for microorganisms exposed to such gases as ethylene oxide because of the complex interaction of heat, concentration of gas, and relative humidity.

D values are estimated for gas sterilization when it is possible to keep heat and humidity values constant, varying only the concentration of gas.

Other key terms used in the determination of microbial death rates include *microbial load,* or *bioburden;* the Z *value;* the *F value;* the F_0 value; and the probability of nonsterility. These terms are defined in Table 21-2, and Z value plots are shown in Figure 21-2.

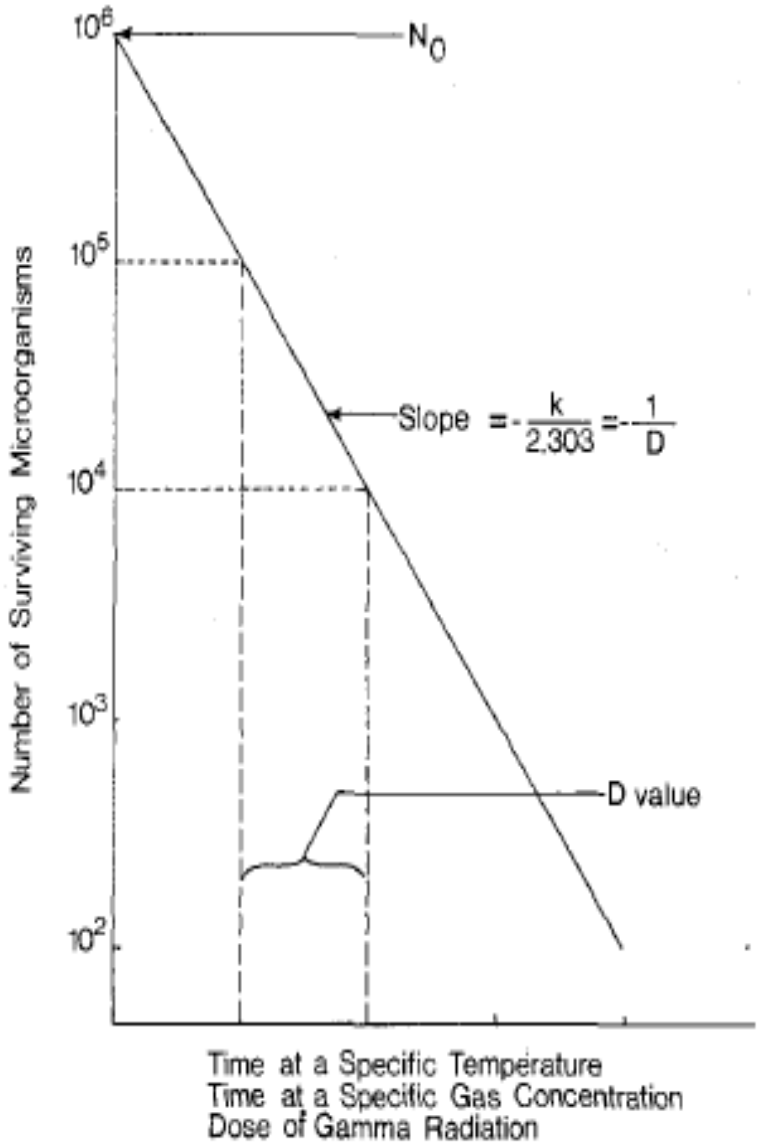
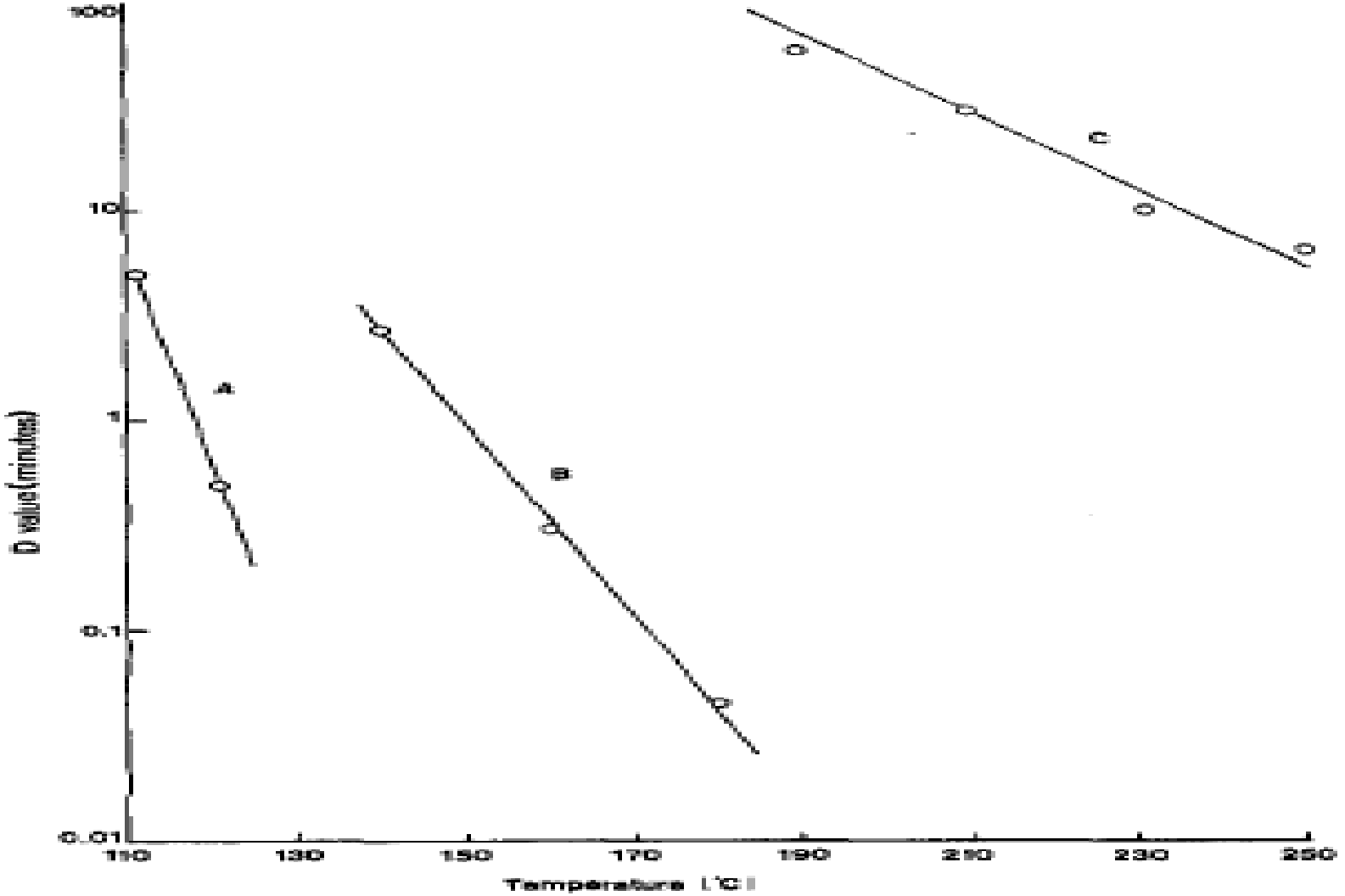


FIG. 21-1. *Graphic representatio:. of the semilogarithmic microbial death rate.*

TABLE 21-2. *Definition of Key Terms Employed in Microbial Death Kinetics*

Symbol	*Term*	*Definition*
N_0	Bioburden	The population or number of living microorganisms per defined unit, surface, or system.
Z	Resistance value	The number of degrees (C or F) required for a 1 log reduction in the D value. $$Z = \frac{T_1 - T_2}{\log D_2 - \log D_1}$$
F (T,z) or F_T^z	Sterilization process equivalent time	The equivalent time at temperature T delivered to a unit of product calculated using a specified value of z.
F_0	Sterilization process equivalent time	The equivalent time at a temperature of 121°C delivered to a unit of product calculated using a z value of 10°C.
N_u	Probability of nonsterility	The number of nonsterile units per batch or the theoretic or extrapolated number of living microorganisms per defined unit after a given equivalent heating time U at a specific temperature T.

$$N_u = \text{antilog}\left(\log N_0 - \frac{U_T}{D}\right)$$



$$Z = -\frac{T_1 - T_2}{\log D_2 - \log D_1}$$

FIG. 21-2. *Z value plots of log D versus temperature.*
Key—A: $Z = 10°C$ *for* B. stearothermophilus *spores exposed to steam sterilization.*
B: $Z = 22°C$ *for* B. subtilis *var* niger *spores exposed to dry heat sterilization.*
C: $Z = 54°C$ *for* E. coli *endotoxin exposed to dry heat sterilization.*

The F_0 value is a term widely used in sterilization cycle design and validation. Its current application is limited to steam sterilization although an F value can be computed for any thermal method of sterilization. The F_0 value can be defined by the following two equations:

$$F_0 = \Delta t \sum 10^{\frac{T-121}{10}} \qquad (2)$$

Where Δt is the time interval between product temperature measurements T.

$$F_0 = D_{121} (\log N_0 - \log N_u) \qquad (3)$$

where N_0 and Nu are those terms defined previously.

The F_0 value of equation (2) is obtained by physical measurement of product temperature and substitution of that temperature for T in the exponent. For example, if the product temperature was measured every 5 min from 0 to 30 min and found to be 25°C, ll0°C, ll8°C, 120°C, 121°C, and *100°C,* the F_0 value would be:

F_0 = 5 min (0 + 0.079 + 0.501 + 0.794+ 5.000 + 0.0079)

$$F_0 = \Delta t \, \Sigma \, 10^{\frac{T-121}{10}} \qquad (2)$$

F 0 = 5 min (6.382)

F0 = 31.91 min

By definition, when the F_0 value is used, the Z value is assumed to be 10°C. This means that for every 10°C increase in product temperature, the D value is decreased by 90%, or 1 log unit.

Equation (3) is the biologic F_0 equation because the F_0 value is calculated after determining the D121 value and the product bioburden, N0.

The probability of nonsterility is whatever level is desired, usually a minimum of 10^{-6}.

In general, equation (3) is applied under two circumstances. Given D121, N_0, and Nu, the F_0 value is calculated. For example, if D121 =1 min, N0 = 10^2, and Nu = 10^{-6},

then: $F_0 = 1 \text{ min } (\log 10^2 - \log 10^{-6})$

$F_0 = 8$ min

Given D121 , N0, and F0, the achieved level of nonsterility may be calculated. For example, if D121 = 2 min, $N_0 = 10^2$, and $F_0 = 8$ min, then:

Nu =antilog $(\log N_0 - F_0 / D_{121})$

Nu =antilog $(\log 10^2 - 8/2)$

Nu = 10^{-2}