This article summarizes and clarifies terms and issues related to the vacuum integrity testing of lyophilizers.

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The Vacuum Integrity Testing of Lyophilizers

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Introduction

Referencing equipment that manufactures Large Volume Parenterals (LVPs), the current Good Manufacturing Practices (cGMPs) state that: "Equipment shall be constructed so that contact components, including process materials, drug products, or the drug product contact area of containers or closures, shall not affect the safety, identity, strength, quality, or purity of the Large Volume Parenteral drug product."[1] Because of the nature of the vapor pressure of ice, both the primary and secondary drying phases of the lyophilization cycle must take place in a vacuum in order to effect the sublimation and desorption of water or other solvent out of the product. In turn, because the lyophilization process occurs in an evacuated vessel, both designers and users of lyophilizers are presented with unique challenges in maintaining the sterility of the product in a vacuum. Among these challenges are the measurement of system "tightness" and the establishment of an inleakage criterion that maintains a reasonable assurance of product sterility. With this in mind, the Vacuum Integrity Test is an important part of any Factory Acceptance Test (FAT), Site Acceptance Test (SAT), and/or Operation Qualification (OQ).

Figure 1. A Typical research lyophilizer.

Basic Definitions

Before exploring practical issues, some basic definitions are essential. One can measure the relative tightness of evacuated vessels by one of two criteria: "rate of rise" or "leak rate." Rate of rise is the amount of pressure change in an evacuated vessel over a given period, e.g., milliTorr per minute (mTorr/min) or milliBar per second (mBar/sec).[2] For example, if one evacuates a vessel to 100 mTorr (0.133 mBar), closes the isolation valve to the vacuum pump, and then observes that after one minute, the pressure is 102 mTorr (0.136 mBar), then the rate of rise is quite simply 2 mTorr per minute (0.003 mBar/min). Mathematically the formula is:

$$\textbf{Rate of Rise} = \frac{\text{Finish Pressure - Start Pressure}}{\text{Elapsed Time}}$$

However, rates of rise, no matter how carefully done, are not an accurate basis for comparing tightness among vessels of various sizes. This is because rates of rise do not account for the volumes of the vessels in question. If a 10 ft^3

Volume: Liters	Volume: Feet^3	mBar/ minute	mTorr/ minute	Volume: Liters	Volume: Feet^3	mBar/ minute	mTorr/ minute
50	1.77	1.20E-02	9.023	3600	127.12	1.67E-04	0.125
60	2.12	1.00E-02	7.519	3700	130.65	1.62E-04	0.122
70	2.47	8.57E-03	6.445	3800	134.18	1.58E-04	0.119
80	2.82	7.50E-03	5.639	3900	137.71	1.54E-04	0.116
90	**3.18**	**6.67E-03**	**5.013**	**4000**	**141.24**	**1.50E-04**	**0.113**
100	3.53	6.00E-03	4.511	4100	144.77	1.46E-04	0.110
200	7.06	3.00E-03	2.256	4200	148.31	1.43E-04	0.107
300	10.59	2.00E-03	1.504	4300	151.84	1.40E-04	0.105
400	14.12	1.50E-03	1.128	4400	155.37	1.36E-04	0.103
500	**17.66**	**1.20E-03**	**0.902**	**4500**	**158.90**	**1.33E-04**	**0.100**
600	21.19	1.00E-03	0.752	4600	162.43	1.30E-04	0.098
700	24.72	8.57E-04	0.644	4700	165.96	1.28E-04	0.096
800	28.25	7.50E-04	0.564	4800	169.49	1.25E-04	0.094
900	31.78	6.67E-04	0.501	4900	173.02	1.22E-04	0.092
1000	**35.31**	**6.00E-04**	**0.451**	**5000**	**176.55**	**1.20E-04**	**0.090**
1100	38.84	5.45E-04	0.410	5500	194.21	1.09E-04	0.082
1200	42.37	5.00E-04	0.376	6000	211.86	1.00E-04	0.075
1300	45.90	4.62E-04	0.347	6500	229.52	9.23E-05	0.069
1400	49.44	4.29E-04	0.322	7000	247.18	8.57E-05	0.064
1500	**52.97**	**4.00E-04**	**0.301**	**7500**	**264.83**	**8.00E-05**	**0.060**
1600	56.50	3.75E-04	0.282	8000	282.49	7.50E-05	0.056
1700	60.03	3.53E-04	0.265	8500	300.14	7.06E-05	0.053
1800	63.56	3.33E-04	0.251	9000	317.80	6.67E-05	0.050
1900	67.09	3.16E-04	0.237	9500	335.45	6.32E-05	0.047
2000	**70.62**	**3.00E-04**	**0.226**	**10000**	**353.11**	**6.00E-05**	**0.045**
2100	74.15	2.86E-04	0.215	10500	370.76	5.71E-05	0.043
2200	77.68	2.73E-04	0.205	11000	388.42	5.45E-05	0.041
2300	81.21	2.61E-04	0.196	11500	406.07	5.22E-05	0.039
2400	84.75	2.50E-04	0.188	12000	423.73	5.00E-05	0.038
2500	**88.28**	**2.40E-04**	**0.180**	**12500**	**441.38**	**4.80E-05**	**0.036**
2600	91.81	2.31E-04	0.174	13000	459.04	4.62E-05	0.035
2700	95.34	2.22E-04	0.167	13500	476.69	4.44E-05	0.033
2800	98.87	2.14E-04	0.161	14000	494.35	4.29E-05	0.032
2900	102.40	2.07E-04	0.156	14500	512.01	4.14E-05	0.031
3000	**105.93**	**2.00E-04**	**0.150**	**15000**	**529.66**	**4.00E-05**	**0.030**
3100	109.46	1.94E-04	0.146	16000	564.97	3.75E-05	0.028
3200	112.99	1.88E-04	0.141	17000	600.28	3.53E-05	0.027
3300	116.53	1.82E-04	0.137	18000	635.59	3.33E-05	0.025
3400	120.06	1.76E-04	0.133	19000	670.90	3.16E-05	0.024
3500	**123.59**	**1.71E-04**	**0.129**	**20000**	**706.21**	**3.00E-05**	**0.023**

Table A. Equivalent rates of rise of given volumes for a leak rate of 1 × 10-2 mBar-L/sec.

(2831 L) vessel and a 100 ft^3 (2831 L) vessel have the same rate of rise, a greater amount of gas must leak into the 100 ft^3 vessel to raise the pressure the same amount, in fact, 10 times as much. To do an accurate comparison, therefore, one must account for the respective volumes of the vessels. This is accomplished by a "leak rate." Obtaining a leak rate involves multiplying "rate of rise" by the system volume. Thus, if a rate of rise is expressed in millitorr per minute (mTorr/min.), then a leak rate is expressed as millitorr × cubic feet per minute (mTorr-ft^3/min.) The general formula is:

$$\textbf{Leak Rate} = \frac{(\text{Finish Pressure} - \text{Start Pressure}) \times \text{Volume}}{\text{Elapsed Time}}$$

or

$$\textbf{Leak Rate} = \text{Rate of Rise} \times \text{Volume}$$

For example, assume that vessels of 10 ft^3 and 100 ft^3 both are evacuated to 100 mTorr (0.133 mBar) and are maintained at a constant temperature. At this pressure, the 10 ft^3 vessel will contain 0.00132 standard cubic feet (SCF) (0.037 L) of gas and the 100 ft^3 vessel will contain 0.0132 SCF (0.37 L) of gas. Assume further that each vessel has an identical leak that allows 0.001 SCF (0.028 L) of gas in one minute into each vessel. At the end of one minute:

- The 10 ft^3 vessel contains 0.00232 SCF (0.066 L) of gas and is at a pressure of 176 mTorr (0.235 mBar) for a rate of rise of 76 mTorr/min (0,101 mBar/min).

- The 100 ft^3 vessel contains 0.0142 SCF (0.40 L) of gas and is at a pressure of 107.6 mTorr (0.143 mBar) for a rate of rise of 7.6 mTorr/min (0.0101 mBar/min).

Both chambers have the same leak yet the smaller chamber has the greater rate of rise. However, if the rates of rise are multiplied by the respective chamber volumes, one obtains:

10 ft^3 × 76 mTorr/min = 760 mTorr-ft^3/min
(283.1 L × 0.101 mBar/min = 28.6 mBar-L/min)

and

100 ft^3 × 7.6 mTorr/min =760 mTorr-ft^3/min
(2831 L × 0.0101 mBar/min = 28.6 mBar-L/min)

The vessels have identical leak rates. Even though the 100 ft^3 vessel has 10 times the evacuated volume of the 10 ft^3 vessel, as long as the vessels are at the same pressure and have identical leaks, virtually the same amount of gas will enter into each vessel over a limited range. This is because the orifice of each leak "sees" approximately the same suction.[3] The obvious advantage of leak rate over rate of rise is that those who own lyophilizers of various sizes can specify a single master acceptance criterion (although the actual test requires that one measure a rate of rise). Figures 1 and 2 of research and production lyophilizers respectively, show just how size can vary among systems. Yet, despite their size differences, both systems can reasonably be held to the same leak rate criterion.

Testing for Vacuum Integrity

The actual testing for vacuum integrity is the same time straightforward and not so straightforward. It is straightforward in that the basic test sequence is simple: chill condensing plates (to protect vacuum pumps), evacuate system, stop evacuation, allow system to stabilize, and measure rate of rise. It is not so straightforward for several reasons: the problem of "real leaks" and "virtual leaks," the influence of system temperature, and the lack of an industry-established acceptance criterion.

Real Leaks

Real leaks can be difficult to locate, but once located often are fixed easily. Location of leaks can be done with equipment as sophisticated as a Helium Leak Detector, or simply by pressurizing the system, coating seal surfaces with soap, and watching for bubbles (although some seals that leak under vacuum may not leak under pressure).[4] On external condenser systems with a main vapor valve, one can close this valve and isolate the chamber from the condenser, and check each vessel for leaks separately. Multiple stoppering rod ports of some older freeze dryers are a notorious source of real leaks. Other common points for inleakage include door seals, main vapor valve flanges, instrumentation connections, thermocouple leadthroughs, relief valves, and process valves.

Virtual Leaks

A major concern for those performing vacuum integrity tests is the presence of what are called virtual leaks. As the name implies, virtual leaks are not real or actual leaks caused by a breach in the vessel's walls or seals. Outgassing materials or gas pockets contained within the vessel can cause a greater rate of rise than one would otherwise obtain. In such a case, one can be led to believe that there is a defect in the vessel's physical structure when in fact there is not. One indication of virtual leak is a decrease in the rate of rise over time. As Figure 3 illustrates, when a virtual leak is present, the rate of rise will taper off as time progresses.

One cause of virtual leaks is humidity and/or fluids within the vessel. If the vessel to be tested is not clean, dry and empty, pressure increases caused by the vaporizing of water and/or solvents (such as from cleaning) contained within the vessel can occur. As the fluids vaporize, the pressure within the vessel increases at least in part owing to the vaporization and not because of any real problem with the system. Water trapped in the chamber and/or condenser drain is a very common source for this type of virtual leak. As the system pressure decreases, water trapped in the drain (upstream of the isolation valve) begins to evaporate. However, the process of evaporation requires energy. This energy comes in the form of a temperature reduction of the standing water, a phenomenon called "evaporative cooling." If enough energy leaves the standing water, the water will freeze, and cause a virtual leak as it slowly sublimates. One field technician's trick to detect this problem is to feel the drainpipe. If the pipe is rather cold to the touch, then one likely has water in the drain.

Second, the outgassing of volatiles from polymers and/or other substances can have a similar effect. As in the first case, volatiles will leach out of polymers (such as seals) until the vapor pressure of the volatile equalizes with the system pressure.

A third type of virtual leak occurs when air (or other gas) is trapped in an annular space that has no opening to the outside of the vessel and a relatively small opening to the inside (e.g. a cavity within a weld). While the main vessel

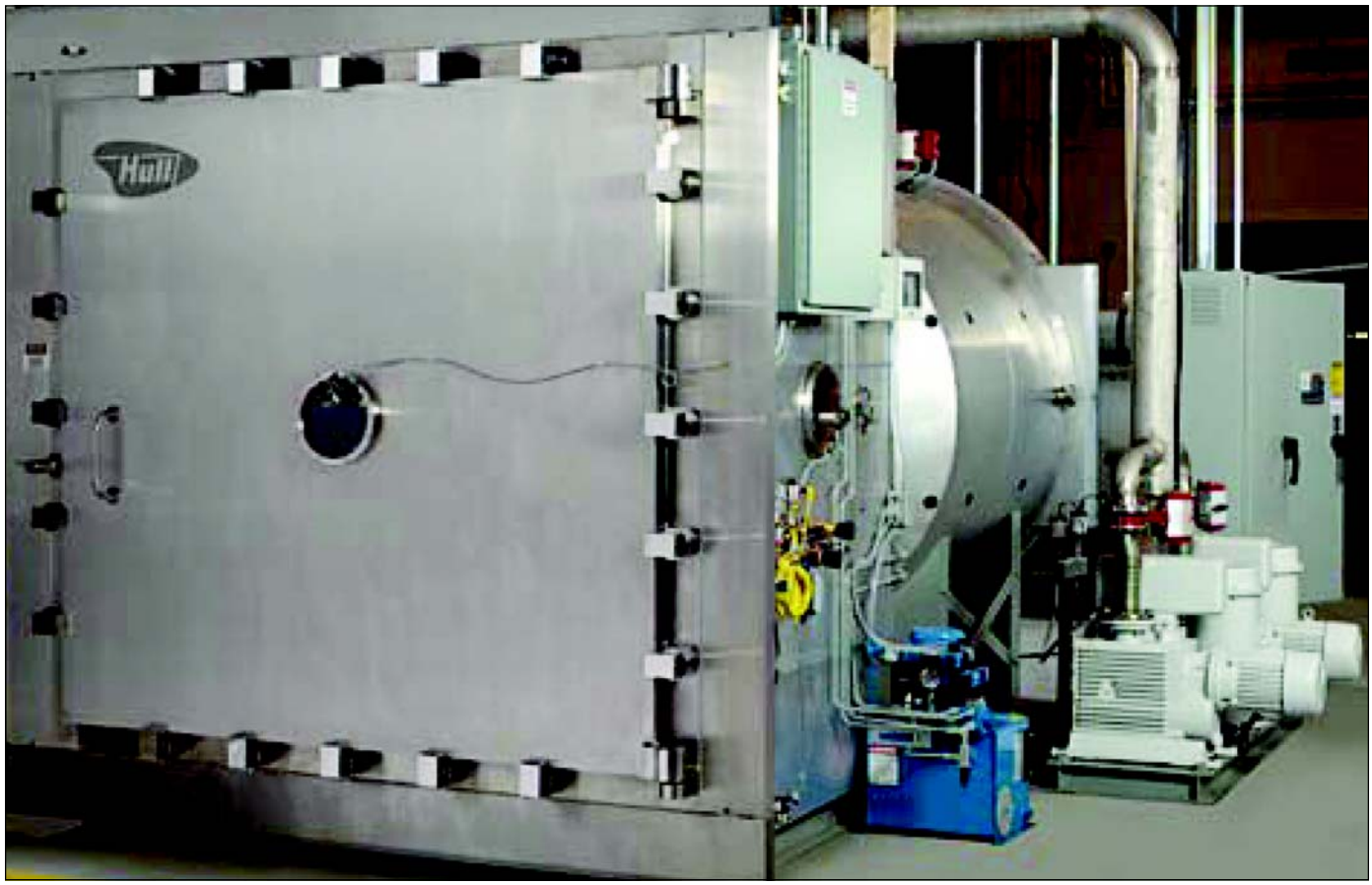

Figure 2. A Typical production lyophilizer.

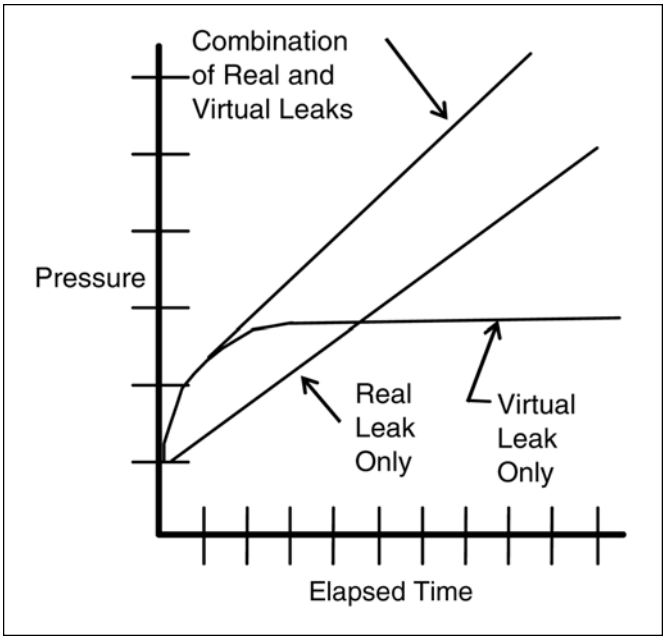

Figure 3. Real and virtual leak plots.

evacuates rather quickly, the gas trapped in the annular space evacuates much more slowly. Thus, while the vessel will appear to have been evacuated to the desired pressure, higher pressure gas will remain trapped in the annular space. When a leak rate or rate of rise measurement is attempted, a false reading will occur because of the gradual leakage of the gas from the annular space into the main vessel.

A properly constructed vessel, especially a vessel certified for positive pressure, should not have any voids, particularly in the welds. In addition, seals made of polymers with vapor pressures lower than the process parameters should be used. EPDM, silicone, and viton work well in vacuum applications and also withstand exposure to steam during a sterilization cycle. Still, only trial and error experimentation can determine if virtual leaks are present. If one suspects a virtual leak, a possible remedy is to evacuate the vessel for an extended length of time. This will allow some vapors to be driven off outgassing substances and/or time for gases to evacuate from annular spaces.

Temperature, Pressure, and Time Considerations

The combined gas law (PV=nRT) tells us that temperature and pressure are inextricably related. Because a system evacuated to the freeze drying range (50 to 300 mTorr, or 0.067 to 0.40 mBar) contains so little gas, and the unit of measure (mTorr or mBar) is so small, small fluctuations in system temperature cause significant variations in readings and results. Fortunately, lyophilizers have shelves with controllable temperatures and condensing plates, which if operating properly, will bottom-out at a consistent temperature (about -95°F (-70°C) for two-stage systems using refrigerant R507). England's Parenteral Society recommends that freeze-dryer shelves be maintained at +104°F (+40°C) to encourage outgassing while the condenser is kept at -40°F (-40°C) or colder to protect the vacuum pumps.[5] Common practice in the United States is to keep the shelves at or below ambient (68°F or 20°C) while allowing condensing plates to attain their minimum temperature.

One caveat, the lyophilizer's refrigeration system can mask virtual leaks. If a surface within the evacuated vessel is cold enough such that outgassing volatiles condense onto it, the effect of a virtual leak can be reduced if not completely abrogated. (Most of the components of air, except water vapor, are non-condensable. As such, the refrigeration system minimally affects real leaks.) Foremost, as long as one maintains consistent temperatures from test to test, one will have comparable results. Furthermore, it is inaccurate to compare the leak rate of a vessel performed without refrigeration to the leak rate of vessel performed with refrigeration.

The pressure at which one performs a Vacuum Integrity Test is also a critical parameter. Rates of rise can be performed at any pressure below the local ambient pressure and can be done for any length of time. The best pressure at which to test is at the expected working pressure of the vessel, usually 100 mTorr (0.133 mBar) for lyophilizers. Specifying start pressures well below that of the system's normal operational parameters is unnecessary and potentially costly for several reasons. Components that satisfactorily contain vacuum at the operating condition can fail at the test condition. In addition, volatiles in substances that do not outgas at the operating condition may do so under the test condition. As such, one can expend large amounts of time, money, and effort attempting to solve a "problem" which does not exist at actual operating conditions. Furthermore, lower pressures cause a greater suction through leaks than higher pressures. Therefore, one should expect lower leak rates and rates of rise at lesser vacuums (higher pressures) and higher leak rates and rates of rise at higher vacuums (lower pressures). In fact, one can obtain a rate of rise or leak rate of "0" with any chamber at local ambient pressure.

Time is the third critical factor. In most cases, the longer the elapsed time, the more assurance one will have of obtaining an accurate result. This is especially true for very tight systems. In such systems, the rate of rise can be so slow as to be beyond the measuring accuracy of even a vacuum head with a 1 mTorr resolution. Rate of rise times of one hour or longer allow the measurement of start and end pressures with increased accuracy.

What is an Acceptable Inleakage Criterion?

First, one must verify whether the leak rate specification is for a complete assembled system or for the individual post-fabricated, but pre-assembled chamber or condenser. An assembled system has many more surfaces to which water can cling, as well as more seals exposed to the surroundings. Second, leak rates are most commonly specified in units of milliBar × Liter per second (mBar-L/sec). The Parenteral Society specifies a leak rate of 2×10^{-2} mBar-L/sec "for a new, clean empty freeze dryer."[6] The current, most frequently specified leak rate for new laboratory and production dryers is 1×10^{-2} mBar-L/sec (see Table A for equivalent rates of rise

for given volumes for this leak rate). This author has found acceptance criteria in practice as high as 15 mTorr/min for a mid-sized freeze dryer. Assuming a system volume of 3,000 liters, this translates to a leak rate of 1 mBar-L/sec or, in other words, a tightness spec 100 times that of the current standard for new lyophilizers.

Yet, experience shows that even lyophilizers with leak rates as high as 1 mBar-l/sec apparently produce product with an acceptable sterility. There are several likely reasons for this. First, because the various molecules that make up air are orders of magnitude smaller than microorganisms, one can have inleakage without contamination. If a system has multiple leaks all of whose paths are less than the diameter of a microorganism, one could have a relatively high leak rate, but still have sterility. Second, leaks through the chamber door seal from a sterile core are inconsequential as long as the leaks are not so large as to prevent a system from obtaining the required process vacuum levels. Third, because the lyophilization process involves the outflow of vapor from the vials, it is statistically improbable that a microorganism would flow "backwards" into a vial. Such an occurrence is even more improbable if the leak is at some point in the vapor path downstream from the vials. Finally, one might observe that larger systems are inherently more sterile because there is more volume to "soak up" microorganisms.

Nonetheless, there is a glaring lack of scientific justification for any of the aforementioned numbers. The Parenteral Society gives no rationale for its number of 2×10^{-2} mBar-L/sec. The current standard of 1×10^{-2} mBar-L/sec for new lyophilizers ostensibly came about as a reasonably obtainable minimum. To determine a leak rate that absolutely would prevent the ingress of microorganisms, one must first consider that potential contamination can occur only if a system has at least one leak path that is large enough to pass a microorganism. The only possible guarantee that no microorganism could enter a system is to test to a leak rate that one would obtain for a single leak path orifice, slightly smaller than the smallest undesirable microorganism.[7] Still, even upon calculation of this inleakage rate, it remains difficult to determine whether one has multiple small leaks, each of which is too small to allow the passage of a microorganism, or some smaller amount of larger leaks, each of which is of sufficient size to pass a microorganism.

Conclusion

- The Vacuum Integrity Test is an integral part of the quality assurance of lyophilized parenterals.
- Nonetheless, there are many factors of which one needs to be aware when performing this qualification, such as the influences of time, temperature, start pressure, and virtual leaks.
- To compare vacuum integrity of vessels, one must have the same temperature, pressure, and time conditions. If the volumes of the vessels are dissimilar, then one must specify a volume-based leak rate.
- Current criteria for acceptable vacuum tightness have not been scientifically justified; however, current practices apparently yield acceptably sterile product.

References

1. 21 CFR Part 212 §67.
2. A common vernacular equivalent to milliTorr is "micron." However, "micron" is ambiguous because it also can refer to 1/1000th of an inch. A milliTorr is 1/760,000th of a standard atmosphere and is the unit most commonly indicated on new lyophilization equipment in the United States. On the other hand, the International Society for Lyophilization - Freeze Drying has issued a standard that calls for vacuum units to be specified in Pascals (Pa). See http://www.islyophilization.org/Html/Standards_Report.html, September 2, 2003. See also Thomas A. Jennings, "Standard Leak Rate for a Freeze-Dryer," *Insight*, June 2000, Vol. 3, No. 6.
3. In theory, however, the smaller vessel in the example likely would see a slightly lower leak rate because its pressure rises more quickly, and thus, its "suction" through the leak reduces more quickly.
4. Of course, one must never exceed the pressure rating of the vessel.
5. The Parenteral Society, *Technical Monograph No.7: Leak Testing of Freeze Dryers*, (Wilshire, England: The Parenteral Society, 1995), 7.
6. *Technical Monograph No. 7*, 9.
7. As a favor to this author, Dr. Narlin Beaty, Chief Technical Officer for Chesapeake Biological Laboratories in Maryland, calculated that a 0.2 micrometer orifice (the standard orifice for sterile filtration), with one atmosphere (760 Torr) on one side and full vacuum on the other, will pass approximately 1.51×10^{-10} moles of air per second at 68°F (20°C) or 7.7×10^{-9} ft3/min ($2{,}18 \times 10^{-7}$ L/min).

About the Author

Charles D. Dern, PE, is a Development Application Engineer for SP Industries (Hull and Virtis Freeze Dryers). He has more than 16 years of experience in all aspects of the design of pharmaceutical lyophilizers. He received his BS in mechanical engineering from Drexel University, located in Philadelphia, and has been a Licensed Professional Engineer in the commonwealth of Pennsylvania since 1993. In addition to his membership in ISPE, he is a member of the American Society of Heating, Refrigeration, and Air-Conditioning Engineers (ASHRAE), and the International Society for Lyophilization - Freeze-Drying (ISL-FD). Dern has lectured on fundamentals of lyophilization and performed customer training on freeze dryer operation at locations throughout the US and Puerto Rico and has been invited to speak in Europe. He has authored numerous "Tech Briefs" for SP Industries' Hull division Web site on various topics such as leak rate, surface finishes, and cold loading considerations. In addition, he shares a US patent for the invention of the "Rotary Disk Valve," developed to replace butterfly and poppet-type main vapor valves used in large lyophilizers. He can be contacted by e-mail: chuck.dern@spindustries.com.

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