Filter Integrity Testing in Liquid Applications, Revisited

Part II

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The authors discuss the influences of test variations, particularly filter inhomogeneity in the characterization of 10-in. cartridges by 47-mm disk testing, and how the use of safety margins can accommodate variances of 10% in the test results. The need for larger test filters is indicated and the advantages of automated test machines are made evident. The authors also discuss “sterilizing” filters and the unsuitability of identifying them by integrity testing alone and urge better bioburden management, not more testing of finished preparations.

Diffusive-airflow imprecisions

Multipoint diffusive-airflow measurements. The diffusive-airflow curve, plotted from readings at several points of increasing pressure, presents a straight line of constantly increasing slope until it begins to curve upward in response to anisotropic pore influences (see Figure 1). Somewhere on the curved section lies the bubble point. The upward sweep of the curve continues until it forms a steep, straight line representing bulk airflow. The curve from its origin as a straight line to the bubble point on its nonlinear section describes the total diffusive airflow. Such a curve may uniquely characterize each of the various types of microporous membranes. Once the diffusive-airflow curve for the filter type has been defined by multipoint assay ing, single-point diffusion testing can, on the basis of this curve, be used to probe the integrity of individual filters of this type. If the single test reading for the filter being assayed falls on the archetypal line, the tested filter is integral. If it falls above the line, the reading is interpreted as signaling the upward curve of a membrane having a lower bubble-point value. That filter fails the integrity test. If the test reading falls below the line at the pressure level tested, most likely a thicker membrane or one of a lesser total porosity is denoted.

If the master curve for the filter type has not been established by multipoint testing, only an empirical correlation exists for judging filter integrity from a single-point diffusion reading. In the case of the bubble point, a correlation substantiated by theory has been demonstrated to exist between its values and organism retentions. Bubble points reflect the
filters’ largest pores and do, therefore, connect with retentions. A causal relationship, such as the one that exists between the bubble point and retention, is required to accept that a correlation exists between events that could otherwise be parallel but independent occurrences. According to Fick’s law of diffusion, diffusive airflows are the consequences of a filter’s total porosity; pore size per se is not an influence (see Figure 2). Thus, no logical connection is immediately apparent between diffusive-airflow values and particle retentions (2). However, although relatively few in number, the larger pores do enable greater rates of flow — flow being proportional to the fourth power of the pore radius or to the square of the diameter, according to the Hagen Poiseuille relationship (3,4). In any case, extending the diffusive-airflow measurement to the bubble-point pressure provides a more reliable reading of the transition point between both airflows.

A degree of inexactness attends the precise location of the bubble point, both in its own measurement and in its determination by diffusive-airflow investigations. However, using both assay methods confines the area of uncertainty and endows it with greater reliability. This is achieved by multipoint diffusive-airflow measurements.

**Single-point diffusive-airflow measurements**

Single-point diffusive-airflow testing enjoys FDA acceptance and has been presumed successful as an appropriate method of integrity testing. It is maintained by some that a correlation must exist between it and organism retention, although the correlation may not be an apparent one: “A filter that has passed a bacterial challenge test will pass a ‘diffusive-airflow’ test, and a filter that has failed a bacterial challenge test will fail a ‘diffusive-airflow’ integrity test” (5). Single-point integrity testing usually is carried out at 80% of the bubble-point pressure. The determination is made as far along the straight portion of the diffusive-airflow line as possible while avoiding the difficulties of measuring on the curved portion (6).

Single-point testing at 80% is based on the assumption that if the testing were performed at multipoint pressures, the diffusive-airflow plot for that particular filter would extend to the bubble-point level characteristic of integral filters of its type. (Stated as a hypothetical proposition in formal logic, the syllogism would read, “If the filter is integral, then it will pass at 80%. It is integral. Therefore, it passes at 80%.” However, affirming the consequence that it passes at 80% does not lead to a valid conclusion, namely, that the filter is integral. Such reasoning suffers the fallacy of affirming the consequence.) Measuring diffusive flows at 80% of the bubble-point pressure cannot demonstrate performance beyond that point (see Figure 1). Nevertheless, single-point integrity testing has had a successful history. It is listed by USP and is accepted by FDA and other regulatory bodies. It is relied upon by many filter users, particularly in Europe. An assumption and the risks inevitably associated with assumptions are, however, involved — an unnecessary risk because the situation can be assessed reliably by multipoint testing without this assumption.

It may be of interest that when single-point integrity testing (forward flow) was introduced, it was intended to be performed at 5 psi pressure. A dependable correlation was judged to be seen between integrity test passage at 5 psi maximum air diffusion and organism retentions. As evident from Figure 3, however, this appeared to be true only because the pressure at which
the filter was tested was below the point where integrity failures, if any, would become evident. Testing at 5 psi could disclose nothing about the filter at pressures above and beyond that level. Integrity failures could manifest themselves all the way up from 5 psi to the bubble-point pressure without being detected (6). Therefore, the protocol for single-point testing was subsequently modified to about 80% of the bubble-point pressure, which is as close as possible to the departure of the curve from its linearity.

It is likely that microporous-membrane manufacture is held to such high-quality standards that flawed product is a rarity. This would account for filters passing at 80%, thereby proving integral. The fact that integrity testing offers evidence of commendable filter manufacturing is encouraging, but the dedicated purpose of integrity testing is to evaluate the specific, individual filter being used. The integrities of its fellows are irrelevant (2). Multipoint testing is preferred for this purpose.

Instances exist in which single-point testing can provide definitive answers. If the single-point testing at the 80% level lies above the straight-line characteristic of integral filters of its type, signaling a higher than maximum allowable rate of diffusion, then the bubble point of that filter is too low, and the filter has failed its integrity check.

Testing above the manufacturer's bubble point. Testing for the passage of air through a wetted filter at pressures above the manufacturer's minimum bubble point could be expected to elicit bulk airflow. Depending, however, upon how high the bubble point of a particular filter is above the minimum for its type, diffusive, not bulk, airflow may still result. This occurrence would attest to the filter's integrity. Filter manufacturers commonly do provide, as an added margin of safety, membranes having bubble points somewhat higher than the minimum levels that correlate with the required organism retentions. In such cases, single-point (forward flow) diffusive-airflow testing is adequate for demonstrating filter integrity.

A single integrity test performed using an automated test machine may require about 20 min. If numerous tests are involved, the time to conduct such tests can be considerably long. The advantage of performing single-point diffusion testing, when it can be applied responsibly, is that it saves time and effort.

The diffusive flow/bubble point combination

The singular significance of the bubble point is that it indicates the largest pores. This is the rationale for its correlation with organism retentions. Regrettably, its exact determination is compromised by the interferences recounted in Part I of this article (1). It is, for instance, not possible to separate the diffusive-airflow component from the bulk airflow. Single-point diffusive-airflows, the product of total porosity, lack the causal relationship peculiar to the bubble point in its dependence on largest pores. However, multipoint diffusive-airflow analyses do present an opportunity for correlation to organism retentions.

Beginning at its origin, the plot depicting diffusive airflows against progressively rising pressures, assayed incrementally to about the 80% level, forms a straight line. Somewhere above this point, it begins to lose its linearity. Hence, it diminishes in reliability, and its implications to retention become vague. The bubble point has its own uncertainty. However, extending the diffusive-airflow testing to include its measurement at the bubble-point pressure restricts the area of uncertainty, thereby limiting and reducing its liability. This makes the bubble-point relationship to organism retentions more certain, which is the goal sought.

Multipoint testing of this sort is suitable for the various initial prefiltration stages of integrity testing. Comparisons may, however, not be made with the postfiltration membrane where sizable particulate deposits have accrued on the filter. The filter thus loaded and altered in its total porosity has, in effect, become a “new,” different filter in its pore structure (i.e., total porosity, pore-size distribution, and size of largest pores). As a result, its diffusion characteristics also have changed. It requires, therefore, its own multipoint plotting for comparison with the prefiltration membrane (7).

Reliable multipoint-testing data, obviating assumptions, can be obtained with as few as two test points instead of the one single-point reading. Measuring air passage at 80% of the bubble point, or at whatever intermediate point is selected, plus measurement at the bubble point suffices. Schroeder indicates that use of the (zero) origin point in addition enables one to draw a straight line on the basis of three points (8). A line plotted from the point of origin through the intermediate test point to the bubble point would affirm the integrity of the filter. However, because the diffusive airflow cannot be separated from the bulk airflow, uncertainty is still introduced into the bubble-point readings. Schroeder suggests that the diffusive-airflow linearity curve should be checked “preferably even slightly beyond [the bubble point] for additional safety margin and to make up for potential inaccuracies in the measurement of the test pressure and the wet flow.”

Advantages accrue to the multipoint defining of the entire diffusive-airflow line, particularly its slope. The slope of the line can be compared with those of other curves. Differences among slopes can have significant meanings. This helps turn comparisons of various diffusive-airflow lines into a diagnostic tool that is useful for probing differences among various membranes.

Figure 3: Diffusive flow through wet filter.
In particular, in the validation effort, it is essential that the water-to-product ratio sanctioning the translation of the minimum water-wet integrity-test value into the minimum product-wet value be valid (9). The water-wet diffusive-airflow line should be compared in its entirety with the product-wet curve. The two lines should be completely congruent. Single-point values simply will not suffice.

Neither the bubble-point test nor the single diffusive-airflow determination by itself serves the purpose of integrity testing as well as do the multipoint analyses. However, once the slope of the product airflow line is at hand, single-point diffusive-airflow testing can be accepted in processing contexts. The likelihood in such cases of a dereliction between the 80% test point and the bubble point is judged acceptably reduced by the fuller characterization of the filter type. Interpretations then can be made on the basis of whether the single-point reading is on, over, or under the diffusive-airflow line characteristic of the filter type.

Automated integrity-test equipment

Automated test machines bring some advantages to the integrity testing practice. Human subjectivity and error in gathering and recording data along with the data's resulting ambiguity are eliminated. This is an important consideration. In addition, unlike the procedure common in manual testing, the garnering of the test information is performed, at least by most of the devices, upstream of the filter. This eliminates the risk to asepsis downstream of the filter barrier. Many automated devices provide a hard-copy printout that can be used as a batch record or as an investigative tool. Some instruments furnish memory cards for data storage or permit electronic batch records to be made via direct connections to the process database system. The automated test machines also can be used to assess the integrity of hydrophobic filters by the water intrusion test (a topic not dealt with in this article).

Upstream measurements are empowered through the use of instruments having data that are fitted to the ideal (or perfect) gas law to enable the necessary calculations to be made. (The ideal gas law may be expressed as \( PV = nRT \), in which \( P \) is the pressure, \( V \) is volume, \( n \) represents the number of moles of gas, \( R \) is the universal gas constant equal to 1.99 L atm/K mole, and \( T \) is the temperature in degrees Kelvin. As for each of the integrity tests, temperature is a variable. It is important that the temperature be kept constant during the performance of the particular test.) The use of mass flowmeters or pressure transducers are principal means of arriving at gas volumes; another method is to use cylinders having precisely defined volumes. The inverse relationship between volume and pressure in the ideal gas law permits the calculation of one from knowledge of the other. As stated, the pressure decay data are translated by way of algorithms, formulae that interrelate the various operative factors into diffusive-airflow data, for which correlations to organism retentions exist.

The several automated integrity tester types may each have its own transition-point standard. Each may define the bubble point somewhat differently. However, the differences among the settings do not seriously detract from the interchangeability of the instruments nor from the usefulness of their measurements.

Variations in bubble points

Sundaram et al. performed an extensive investigation of the effect of test methodology on bubble-point analyses using two manual and four automated protocols involving several types of filters in disk form (47-mm diameter) and in 10-in. cartridge form (10). All of the tests were performed by each of two operators. They concluded that the term bubble point “refers to a collection of many different test methods” being defined variously by different procedures, measured by different means, and having different end points. The different results obtained largely can be explained on the grounds of familiar influences such as the filter area, measurement sensitivity, subjectivity, and filter inhomogeneity. The last-named factor can exert a significant effect on the translation of integrity-test values from small disk filters to membrane cartridges.

The range of measurements showed variations of 5–6 psi, “depending on the exact type test used.” The variation in results encompassed automated as well as manual testing. The point is that unquestioned precision does not characterize even the much vaunted bubble-point test. Its traditional variability of ~10% seems about right. This average variation was found to hold both for 47-mm disks and for 10-in. cartridges (11).

The ~10% variation among measurements does not detract neither from the usefulness of the integrity tests in their filter
Identification function nor in their role as indicators of differences in pre- and postfiltration situations. At their present level of accuracy, the integrity tests can distinguish among the various pore-size identification ratings. They also are capable of disclosing gross filter damage, of indicating subtle incompatibilities between membrane and liquid, and of affirming the integral assembly of the filtration train.

Translation of integrity test values
Sundaram et al. conclude from their studies that “no direct correlation of disk ‘bubble-point’ value to cartridge ‘bubble-point’ value exists, even when the same ‘bubble-point’ type test is used for both determinations. The ‘bubble-point’ value measured for the 47-mm disk used in bacterial validation is not suited as the value for a process-filter cartridge specification even when using an automated integrity test instrument” (11). Therefore, much concern is expressed regarding the translation of the water-wet disk values into those corresponding to the cartridge and subsequently the corresponding product-wet values as well. This translation is a key requirement in the filter validation exercise because organism retention is determined using 47-mm disk filters while the processing is performed with membrane cartridges.

Sundaram et al. found, as did Johnston et al. two decades earlier (12), that the numbers obtained from 47-mm disks are always higher than for cartridges. Therefore, any error in assigning 47-mm-disk integrity-test values to cartridges in defining the identity of the water-wet production filter is at least in the direction of overcorrection. However unintention, it translates to an act of prudence.

It would be worthwhile if filter manufacturers would undertake studies imitative of those conducted by Sundaram and his colleagues, which focus on the unit inhomogeneity of several microporous membranes. The results would help define the smallest filter area suitable for the given membrane type that would be required in organism retention validation studies. As stated previously, filter manufacturers are increasingly assessing the organism retention properties of their filters using larger areas of membrane than the usual 47-mm disks.

Definition of “sterilizing” filter
The defining of the (minimum) integrity-test value indicative of a “sterilizing” filter as one characterized by its ability to accommodate organism challenges of $1 \times 10^7$ cfu of *Brevundimonas diminuta* per square centimeter of effective filtration area is too well known to require elaboration. (Reviews of this area can be found in references 15 and 16.) The erstwhile importance of the integrity tests was that their values were seen to identify such a filter.

Uncertainties do exist in defining the precise (minimum) bubble point indicative of the formal $1 \times 10^7$ cfu/cm² B. diminuta retention, but this always has been the case. Johnston et al. found that the plot of organism retention versus bubble point had a slope of two (see Figure 4) (13). A 10% discrepancy in bubble-point readings would make the difference between retentions of $1 \times 10^7$ and $1 \times 10^5$. Filter manufacturers finesse the situation by furnishing membranes that have bubble points in excess of the minimum “sterilizing” value. The price the filter user pays is a certain lower rate of flow, and, for more heavily loaded liquids, a possibly abbreviated throughput.

Nevertheless, the identification of the “sterilizing” filter with the integrity-test value emphasized the need for precise measurements. The singularity of the proper integrity-test value defined the “sterilizing” filter. Consequently, the positive assurance that such a filter was being used formerly was enough to validate the filtration process. This practice is recognized to have been an oversimplification, particularly as the organism retention mechanism was assumed to depend exclusively on the size exclusion of a sieving action.

To emphasize, the attainment of a sterile filtrate is now understood to be more complex. It depends upon an interaction of several factors, namely, the pore-organism size relationship; its possible modification by contact of both the organisms and filter with the liquid medium; the effect of the liquid’s properties on the electric double layer and the consequences to adsorptions; and the thrust of the filtration conditions, especially the hydraulic influences that govern organism captures by adsorption. In these circumstances, the role of integrity testing loses its former preeminence. It no longer signifies the reliability of organism arrests that it once presumed.

The situation is one in which integrity-test values, while important, do not have the singular significance they once had in this application. By the same token, the accuracy of their readings can accommodate some leeway. Efforts to refine them can needlessly be exaggerated.

At present, a risk to filter validation may be an overreliance on the integrity-test values. Previously, an erroneous test value could lead to the wastage of the filter and to the discarding of the entire production batch. Clearly the filter is best considered a vital element in a holistic microbial control process rather than the sole guarantor of “sterility.” Validations now should be affirmed by microbiological analyses given direction through bioburden studies (14,15). The misdirected use of a filter that did not meet its performance specifications still would result in the wastage of the filter and of the filtration operation. However, careful process control requirements to ensure well-controlled bioburden can provide a safety level that cannot be ensured by filter system testing alone. This added safety is vital given the uncertain relationship among pore rating, integrity testing, and retention.

Conclusion
The integrity-test procedures now in place satisfactorily disclose filter porosity ratings. They are capable of indicating filter damage, and they reveal even subtle incompatibilities between the filter and fluid. In addition, they provide information regarding the integrity of the filtration train. However, changes exist associated with the role of integrity testing in filter validations, and the limits of membrane homogeneity are better recognized.

The exact integrity-test values by which a filter type in its 47-mm disk configuration currently is defined as a “sterilizing” filter (namely, by its proper retention of B. diminuta) may not directly translate to cartridge filters. This fact has promoted efforts...
to designate with great accuracy the precise datum point so that the transition can be made correctly. Concern also exists that exact integrity-test values may be shaded by interferences arising from the effects of filter area, from the limitations in measurement making, and from other influences. This too has led to attempts, perhaps exaggerated, to accurately define the transition points.

Epic efforts in this direction seem, however, to be unnecessary. Filter manufacturers produce microporous membranes having a safety margin in the integrity test values. These should exceed the minimum bubble points and/or the maximum diffusive airflows sufficiently to avoid the problems. Indeed, this may create problems at the production end where process filters with values close to the minima or maxima, as the case may be, are sought so as not to pay unnecessarily for the security margins in terms of restricted process flows.

In addition, integrity-test values no longer are considered as certifying validation. The attainment of sterile filtration is not so dependent on any integrity test value that it necessitates meeting its numerical level with exquisite accuracy. This denouement seems a difficult one for the industry to accept, so assuring was the former concept of the certainty of validation by integrity testing and so much more complex is the present understanding, particularly as the newly recognized influences are so little comprehended. The reality remains, however.

Indeed, the pharmaceutical industry must reconsider entirely the concept of the “sterilizing” filter. At present, no universal sterilizing filter is known to be capable of arresting all organisms in all circumstances. Such a filter would have to rely totally upon the sieving mechanism of particle removal to avoid the unpredictable occurrences of adsorptions. The sterilizing filter need not be capable of retaining all organisms, known or unknown, under all conditions from any and all media. A realistic definition of a “sterilizing” filter is one that under the given filtration conditions removes all the organisms known or likely to be present, or at least those thought to be important from a user-safety perspective. Filters may yield sterile effluent regardless of their pore-size ratings, provided the filtration conditions are suitable. Therefore, the integrity tests, indicative of pore sizes, lack in this application the ultimate authority they previously held.

Unfortunately, sterilization may have to be defined and detailed on a case-by-case basis with specific considerations of the organism type, the filter type, the constitution of the liquid vehicle, and the filtration conditions. Above all, sterility will require definition in terms of the organisms with which removal is sought. This necessitates bioburden studies. It also may require a more scientifically precise understanding of the terms sterile and aseptic and an acceptance of the fact that some uncertainty is inherent in retention-based microbial control at the current state of technology.
Until technological capabilities dictate otherwise, reliance should continue to be placed on the present system of generally using B. diminuta as the test organism, and the 0.2/0.22-µm-rated membrane as the "sterilizing" filter, as detailed in PDA Technical Report #26, "Sterilizing Filtration of Liquids" (9). The present situation does not call for more testing of finished preparations but for a more suitable management of the bioburden from its very upstream origins.

References