

The Filter Scene: Old and New

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Advances in filtration have paved the way for a more educated laity, an increased emphasis on validation, and a greater knowledge of the limitations of integrity tests.



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During the past 25 years, the pharmaceutical industry has seen several changes in the area of filtration. First of all, filter users have become much more knowledgeable. There is a better informed and educated laity out there. That is a positive development. With that understanding, intellectual challenge has come to the issues and decisions that were once left almost exclusively to filter companies. As a negative, however, where there had been many filter courses given, these are now seldom offered because it's as if "we know all about filtration," which isn't quite true. More is still to be learned by many filter users. At least, however, filter users now add their own understanding to that of the filter purveyor.

This increased knowledge has led to a greater realization for the need of validations. There was a time when to validate a filtration exercise, such as a sterilization process, all one had to do was to rely upon an integrity test to show that a "sterilizing" filter was being employed. Now the industry recognizes, or should recognize, that there is no universal "sterilizing" filter. The 0.2/0.22 μm -rated sterilizing grade filter, although suitable in most pharmaceutical contexts, is not absolute against all organisms. The 0.1 μm -rated membrane, urged by some as a substitute, has similar limitations and has its own shortcomings. Many suspect its advocacy to be a marketing ploy. Nevertheless, it is based on the recognition that the *B. diminuta* organism is not a model for all other microbes. The certainty held before, that certain filters will inevitably give sterile solutions, is now seen not to be so. Achieving sterility may also depend upon the specific organisms involved, how their morphology and that of the filter may be affected by the suspending solution, and how the filtration conditions (e.g., the hydraulic influences, pressure, viscosity, etc.) may influence the organism retention mechanisms, particularly that of adsorption.

Our considerations of the mechanisms of filtration still focus on sieve retention because size exclusion is easy to understand. There seems to be an avoidance of dealing with adsorption because we don't quite know how to handle it. Nevertheless, there is a recognition that filtration is a complex operation, and that it's not enough to try to certify it or validate it by way of integrity tests that are interpreted solely in terms of sieve retention or size exclusion. Validation requires one to examine the final effluent and make an assessment based on microbiological assays and bioburden studies. One must look to the adsorption-influencing effects of the suspending vehicle and

to the filtration conditions. The belief in a universal sterilizing filter whose dependability of operation is based on a pore-size/organism-size relationship is incorrect.

The integrity tests themselves now partake less of a ritual. There is a greater understanding of their implications. Formerly, the different tests were seen by some as being competitive. The question would be, "Which is better, bubble point or diffusive airflow?" Now it is recognized that, although there may often be a choice as to which integrity test to use, circumstances, usually depending upon the effective filtration area of the filter, dictate the choice. There is not a competition between the tests. For small filters, bubble pointing is the indicated method. Where the bubble point would be masked by a sufficiently large diffusive airflow, the diffusive-airflow test must be depended upon. In the early days, most filter companies seemed to prefer the bubble-point tests. There is also the forward-flow test, which is a single-point diffusive-airflow test.

Diffusive-airflow testing, unlike the bubble-point test, reflects the filter's total porosity, not just its largest pores. Therefore, it would seem that single-point diffusive-airflow tests in themselves cannot possibly serve to integrity test a filter. However, one also accepts that on the basis of history, at least in the case of certain filters, the test does seem to be reliable. On a technical basis, single-point diffusive-airflow measurements would seem unjustified, yet they are widely applied despite the risks their assumptions entail.

Actually, the entire problem becomes obviated by the application of multipoint diffusive-airflow testing. The diffusive airflows should be measured at two or three pressure points, including at the bubble-point level. The performance of such a diffusive-airflow test, extending the single-point by only one or two additional measurements, should be the standard practice. The key is the inclusion of the inquiry at the bubble point, for it is the straight-line conjunction of the diffusive-airflow curve with the bubble point that gives authority to the airflow assays.

Increasingly, it is acknowledged that the integrity tests have their limitations. Validations based on bioburden studies are needed. The former belief in absolute filters (absolute nonsense) has been replaced by the acceptance that this is not the case. As we continue to look further, we are bound to uncover other myths, and we will have to continue to make judgments. One had always expected or hoped, I think, for an excessive degree of certainty in sterile filtration — a security that seems to be absent in every other human endeavor (including my investment in the stock market). But while it is recognized that there is no occasion for panic, it is understood that conducting sterilizing filtrations is a serious business, fraught with potential pitfalls. Care must be taken.

What impresses me is that those who, like myself, are not microbiologists, look for the ultimate validation in the microbiological area. Surely, one tests the effluent to see what one has — the proof of the pudding being in the eating. The microbiologists, on the other hand, understanding the limitations to their techniques, look to the membrane specialist for the ultimate answers. The membrane specialist, in looking to the microbiologists, acknowledges that he is dealing with an incomplete and

imperfect situation. Microbiologists, in looking for a solution to come from the filter gurus, by that very token acknowledge that they too don't quite have the answers at their end. Clearly, therefore, more work is needed on both our parts. Future efforts will bring us closer to one another and to a more perfect situation.

In complex systems, one tries first to define what the controlling factors are. We have been doing that more or less successfully and progressively. We still need to know better the interactions among the several factors that govern sterile filtrations. An example of that is the former "matrix method." Its databank served as the validation. A specific experimental validation seemed unnecessary if the right filter were used, as defined by its having successfully experienced the factor(s) and/or condition(s) of the filtration in a previous filtration exercise. That notion, good to relate, went by the board. It was recognized that not just the individual influences but their interactions were controlling, and these were unknown. We now appreciate that the right filter is defined by many influences: by the organism type, by the organism's response to the solution, by the response of the filter itself to the solution, by the hydrodynamic consideration, and by the properties of the suspending fluid. We recognize more the complexity of the process, and I think we are a little more humble. Perhaps we are a bit less certain and, therefore, less arrogant from a technical point of view. We know we have to do more.

Consider the phenomenon of "grow through." About 25–30 years ago we already knew of grow through: Certain organisms might not be able to penetrate a given filter during a filtration, but when the filter in wet condition with the organisms on one side was allowed to remain that way, the organisms appeared on the other side after a period of time. How could that be? One rationalization is that when the organisms reproduce, they undergo binary fission and divide into halves, so their size is smaller. Possibly in that step they work their way through the filter. There is, of course, also a marketing angle. As one filter manufacturer had it, when enough organisms become present through reproduction, they find the few larger pores and make their way through the filter: "They don't penetrate our filters because ours have no large pores. They go through the other guy's filters." In the actual event, the problem is avoided because FDA requires the entire operation, including the filtration, to be completed within eight hours. Grow through takes more time than that to develop, so there is apparently no grow-through problem.

However, the reason for grow through could be important. One explanation relates to organisms characterized by a semi-permeable membrane. One hypothesis holds that it depends upon the hydrophobicity–hydrophilicity mix of this surrounding membrane. In a solution that is hypotonic, water will flow into the organism through the semipermeable membrane. The cell will swell. In a hypertonic solution, water will flow out from the organism into the solution, and the organism will shrink. The singularity of the response of the organisms would be a consequence of their particular hydrophobic–hydrophilic mix, as would also the velocity of the size change. This possible explanation would account for differences in responses among dif-

ferent types of organisms. This possible approach to grow through comes from fields other than the pharmaceutical industry. That's an encouraging situation. We are utilizing information from other fields. Thus, TOC measurements came to us from the semiconductor industry. We are less isolated in technical matters. This explanation of organism grow through comes about from studies in dentistry on biofilm plaques.

In summary: We recognize the greater complexity of our field. I think we have become a little less comforted by oversimplifying assumptions — less strident. We've become somewhat humbled by realizing that there's a much bigger field than we had thought, but we also speak more quietly because there's a greater confidence — we know more. Twenty-five years ago if a filter failed, one called up the filter company and one might hear essentially, "Tell us what you did wrong, and we will correct it." Now we understand that nothing was necessarily done wrongly, nor was the filter necessarily faulty. It's just that there are more interactive influences than had been appreciated.

As for the filtration field of the future, there are technical paths that can lead forward, but which of these will be developed will depend, among other things, upon marketing considerations. Of course, there will also be disappointments. These too may reflect the real world. For example, a pharmaceutical processor validated a given filter listed in its drug application. Then the filter company stopped making the filters. There was no difficulty in manufacturing the filter, and its discontinua-

tion caused negative feelings with the now abandoned user. The filter was discontinued because its manufacture was not supported by sales sufficient to prove profitable; it just didn't pay to manufacture it. The ongoing quest for and fulfillment of advancement and novel breakthroughs can be limited by legitimate market forces. The sharp focus of the technically minded individual may on occasion need reminding of the economic realities of the world.

The elucidation of adsorptive effects will figure prominently in future work. Adsorptions and their management are important factors in the fermentation activities of the biotechnology industry. Enzymes and other protein products can adsorb to filters. Different polymers have different propensities for adsorption; the polymer is not merely an inert matrix for the pores. The polymer can intrude into the filtration process. More can advantageously be learned about adsorption and/or its avoidance.

If there is any pertinent field that is not sufficiently drawn upon by the filter industry, it is surface chemistry. It's amazing how little most filter practitioners comprehend about this discipline. Hydrophobicity, a surface phenomenon, is important because proteins are largely fixed to filters by hydrophobic adsorption. Possibly the same goes for organisms whose surfaces likely feature arrangements of amino acids. Now one can classify filters by their degree of hydrophobicity, and one can characterize protein structures in similar terms. It ought to be possible, therefore, to match or mismatch filters and proteins to promote or to avoid adsorptive captures. If one of a mix of proteins were sufficiently different in hydrophobicity from that of the other proteins in the mix, then one would be able to separate it using a filter of a known and proper degree of hydrophobicity. We do know that different organisms adsorb differently to different polymers.

Proteins, like all extensive molecules, exist in coiled configuration, the degree of which depends on their extent of dilution or upon the ionic strength of the solution. According to the Fuoss effect, increased coiling results in the greater exposure to the aqueous solution of those constituting amino acids of the protein that are most hydrophobic, namely, tryptophane, tyrosine, and phenylalanine. The hydrophobicity of a protein in solution being an expression of its degree of dilution is an added consideration to the above. Whether this offers opportunities or presents problems remains to be seen. Nevertheless, the means may exist, based on hydrophobic adsorptive sequestrations to filters, whereby protein separations from complicated compositions may become managed.

It will be interesting to see what we can say about our filtration views 25 years from now. Join me; I plan to stick around and see! **PT**