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Particle Retention Testing of 0.05 to 0.5 Micrometer Membrane Filters*

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Key Words:

- Polystyrene
- Fluorescent Microspheres
- Particle Retention
- Biological Membranes
- Pore Size
- Experimental Procedure

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1. INTRODUCTION

Innovations in the design and use of membrane filters have been a key factor in the quest for better quality electronics, medicines, beverages and biochemicals. As the filtered products[1] and contaminants[2, 3] come under greater scrutiny for both their value and their importance, more attention must be given to meeting and verifying the filter specifications, especially at the smaller pore sizes. This report discusses some historical approaches to membrane filter testing and some new and improved methods for checking retention ratings for 0.05 to 0.5 micrometer (µm) membrane filters.

2. TRADITIONAL METHODS AND CHALLENGE MATERIALS

Retention testing of membrane filters with sub-micrometer pore sizes is best approached by a brief review of some methods and challenge materials historically used and how they relate to present day analytical problems.

Traditional analytical methods include microbiological assay, optical and electron microscopes, automatic particle counters, and various light scattering and turbidimetric systems. Improvements in measurement technology have not been completely successful in meeting the demands of filter manufacturers for more rigorous performance testing at the smaller pore sizes. The basic problem is that the complexity and cost of the measurement methods increases as the pore size ratings decrease. Thus, the filter designer or user has to confront the problem of becoming or hiring a career microscopist or particle analyst to test and verify the filter performance. In order to keep the main emphasis on the filters and not the analysis, there is a definite need for more effective and less costly analytical methods.

The development of improved analysis methods has increased the need for new and better challenge materials. Historical challenge materials used for testing coarser filter ratings include materials such as A/C test dust, pollens, glass beads, and large polymer beads. These materials are unsuitable for testing filters with less than 1 µm ratings. Smaller size challenge materials include such items as microorganisms, dioctylpthalate esters (DOP), colloidal silica, and polystyrene latex particles. Microorganisms such as pseudomonas and mycoplasms have their place in specialized testing applications[4, 5], but their use requires special training, is only semi-quantitative, is limited to certain pore sizes, and does not provide pore-size distribution data. DOP esters are primarily used for testing only one size of aerosol filters and the colloidal silica is too small and polydisperse to be useful for 0.45, 0.2 and 0.1 µm pore-size ratings.

Of the various challenge materials, only polystyrene latex particles offer the potential for testing both the retention value and the size distribution of a broad range of sub-micrometer pore sizes. However, there are limitations to the use of polystyrene latex particles, imposed primarily by the various analytical methods used to detect and measure them. For example, electron microscopy is normally so limited by its cost and complexity that it is usually relegated to research uses, rather than routine testing. In addition, only a few particles out of the millions used in the test could be analyzed, raising questions of sampling error. Sample collection and preparation are complicated and the analytical turn around time is excessive. Scanning electron microscopes offer some improvements in operator efficiency, but the necessary sputtering of the sample may alter the diameter of the spheres and add artifacts. Optical microscopes are less expensive and easier to use, but require skilled operators and are not much use for sizing or counting particles smaller than 0.5 µm.

Automatic particle counters which both count and measure the particles by means of electrical resistance, sedimentation, or laser light scattering systems are typically rather expensive and require a great deal of time and expertise to operate properly. They usually offer marginal performance for analyzing polystyrene particles less than 0.5µm in diameter[6, 7]; however, newer models of laser light scattering systems under development have the potential for analyzing 0.1 µm liquid borne particles[8].

The most cost effective and practical methods for analyzing suspensions of polystyrene latex over a wide range of sub-micrometer sizes are the various light scattering methods such as turbidimeters, nephelometers, colorimeters, and spectrophotometers. The advantages and limitations of these methods will now be described in more detail.

3. LIGHT SCATTERING OF POLYSTYRENE LATEX PARTICLES

Direct measurement of light scattering of suspensions of uniform polystyrene spheres is an effective form of detection for these challenge materials. The particles will scatter a significant amount of light at relatively low concentrations, allowing a direct determination of the particulates transmitted by the filter. The measurement instruments vary in the monochromatic or polychromatic nature of the incident light, the geometry of the light source, and the angle and design of the detection system, but the basic principle is essentially the same. Incident light is scattered by the particulate dispersion and the attenuation is monitored by a photo detection system.

In the case of light-attenuation measurements (caused by light scattering) in a conventional spectrophotometer, the method is limited by the concentration of the filtrate. High concentrations can be analyzed via serial dilution after a linear response curve is established. After developing a light scattering procedure for filter testing, the sample preparation and analysis can be easy and rapid. Modest technical training is needed to run this form of test compared to tests using more complicated instruments such as electrical resistance counters and electron microscopes.

Figures 1 and 2 show the data for light scattering at several monochromatic wavelengths across a wide range of diameters. Clearly both illumination and diameter significantly affect the observed scattering.

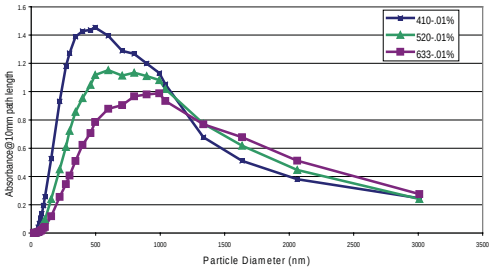

Figure 1. Light scattering of Polystyrene Latex Particles at 0.01% wt/vol concentration

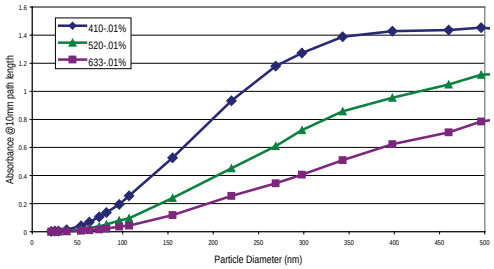

Figure 2. Light scattering of Polystyrene Latex Particles at 0.01% wt/vol concentration

The sensitivity of light scattering methods generally falls off significantly below 0.1-0.2 µm particle diameters. This is due to the fact that at lower sizes, particles have extremely low volumes and highly curved surfaces, so appreciably less light is scattered at longer wavelengths. In larger sizes, about 1 µm, there is little difference in light scattering with wavelength, a red 633 nm laser is as effective as a 320 nm UV analysis. At 0.1 µm however, the 633 nm wavelengths are simply not scattered as much by this size particle as would be wavelengths much closer to the size of the spheres. In these small diameters, a blue or UV source is much more effective in producing detectable scattering signals, subject to one important limitation: any extract that is chemically active at the incident wavelength will produce an artifact within the analysis. The increased signal, when compared with the upstream challenge particles, might be recorded erroneously as transmitted particles. The interfering substances can come from the cuvette, filters, hoses, plastic fittings, etc.

For all light scattering methods, it is important that measures be taken to insure that no foreign particulate is analyzed along with the test contaminant. This is especially difficult in the case of polymeric fragments or fiber from the media. To emphasize the importance of minimizing oversize challenge particles or foreign contaminants, consider that one 1.0 µm particle will scatter the same amount of light as 12,000 0.1 µm particles at the 520 nm wavelength. To minimize the presence of foreign particulates, the filter to be tested should be thoroughly flushed with highly filtered, deionized water before beginning the test.

For an accurate light scattering test, the challenge particles must be uniform and free of agglomerates. Most latex particles from 0.1–0.2 µm are relatively uniform in size, but commercial formulations often produce polymerized larger size droplets as well as populations of near sized particles. Latex suspensions stored or supplied at high percent solids often contain enough high-scattering agglomerates to significantly influence tests where the upstream concentrations are also determined by light scattering measurements.

The challenge material must also be well dispersed so as to minimize capture by mechanisms that are not

related to physical screening by size. A well designed test must therefore either use particles that have been supplied in a well dispersed system or one must be created, preferably one with sufficient non-ionic and ionic surfactant activity to minimize hydrophobic capture, electrostatic trapping, and hydrogen bonding. In the case of light scattering tests, it is important that the same dispersion quality also exist downstream from the filter. A sample that agglomerates in the downstream solution can give significantly higher scattering signals, as might happen if the surfactant is stripped out by hydrophobic media.

A series of polystyrene particles have been developed which meet the criteria for accurate light scattering measurements. They are available commercially as Thermo Scientific Nanosphere Size Standards, 3000 series. For less demanding applications, the 5000 series of latex microspheres is also available.

In summary, light scattering analysis of polystyrene latex microspheres is a good method for membrane filter testing, but test sensitivity falls off rapidly below 0.2-0.3 µm diameters. Methods can be improved by using monosized polystyrene particles dispersed in a medium which will prevent agglomeration or capture of individual particles by mechanisms other than size screening.

4. FLUORESCENCE DETECTION METHODS

Although the light scattering methods work reasonably well, a method was needed which would more decisively distinguish between the challenge particles and background or interfering substances. Fluorescent dyes have been used in other scientific fields for highly sensitive assays, but until now, the method has not been successfully transferred to either filter testing or particle analysis. A series of polystyrene microspheres has been developed as series of monodisperse fluorescent microspheres having red, blue and green fluorescent colors in a range of sizes from about 2 µm down to 0.025 µm. The dyed particles are suspended in aqueous media, and have large shifts between the excitation and fluorescent spectra, as shown in Figure 3. The particle suspensions have been prepared to minimize the presence of particles that are larger or smaller than the main population. The suspensions contain dispersing agents, which minimize filter retention by mechanisms other than particle size. This series of fluorescent microspheres can be used for fluorescence microscopy and fluorescence spectrophotometry applications.

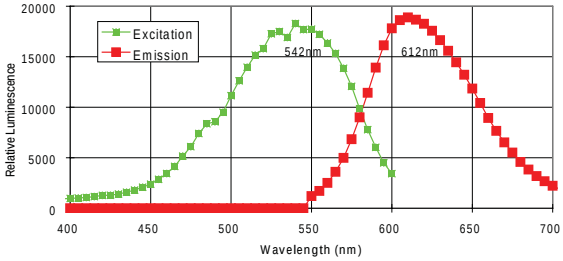

Figure 3. Thermo Scientific Red Fluorescing Particles

5. EPIFLUORESCENCE MICROSCOPY METHODS

An important innovation, which makes practical the use of fluorescent microspheres, is the epifluorescence microscope. It differs from ordinary fluorescence microscopes in that the sample is illuminated from above rather than below. The fluorescent light is then emitted upward from the sample, back through the objective, barrier filter and eyepiece for observation. With this configuration, the incident light does not provide background interference. This permits at least an order of magnitude more sensitivity to fluorescent light than with conventional fluorescence microscopes. Most epifluorescence microscopes have several sets of bandpass and barrier filters to provide convenient changes of fluorescence parameters.

The epifluorescence microscope and the new fluorescent microspheres provide a powerful, yet simple and relatively inexpensive method of membrane filter testing. Fluorescent particles as small as 0.45 µm or 0.3 µm are dramatically easy to observe and, with practice, 0.2 µm and 0.15 µm particles can also be observed. In principle, one uses particles of two different fluorescent colors and two particle diameters. For instance, one particle might be selected with a diameter at or slightly above the filter rating, and be red fluorescing. The other might be smaller than the rating and be blue fluorescing. Examination of the surface of the challenged filter would show the presence of virtually all red microspheres. The filtrate, collected downstream on a membrane of lower pore-size rating, should show mostly blue particles and few or no red particles. The ratio of red to blue particles upstream and downstream can provide semi-quantitative retention values. The main feature of the method is that the fluorescent color is used to indicate the size of the particle, eliminating the requirement for particle measurement.

At the author's laboratories, the epifluorescence microscope and fluorescent microspheres have been used to evaluate membrane filters with ratings of 0.2 µm and larger. The method is easy to use and is without the light scattering limitations imposed by non-fluorescent polystyrene latex microspheres.

6. FLUORESCENCE SPECTROPHOTOMETRY METHODS

These methods use the same series of fluorescent polymer microspheres described for the epifluorescence method, except the fluorescent dyes are measured quantitatively on a fluorescence spectrophotometer instead of being visually observed through a microscope. The dissolution method involves dissolving the polymer spheres and freeing the dye into solution for analysis by a spectrophotometer. The direct method utilizes smaller spheres, which are measured directly in the challenge fluid by the spectrophotometer.

Dissolution Fluorescence Method

In the dye dissolution test, the downstream filtrate is diluted into a solvent for the particles. A suitable solvent is methyl pyrrolidone. The polystyrene particles are fully dissolved in the solvent, which can accommodate a certain percentage of water while retaining sufficient solvency for the polymer. At a typical 1:9 dilution of aqueous fluorescent particle dispersion in the solvent, the dye is freely released into solution and can easily be read by a fluorescence spectrophotometer.

The spectrophotometer readings are correlated with known calibration curves typically generated with serial dilutions of standard samples. For the dissolution fluorescence method, the threshold limit on a moderately priced machine is about 10 parts per billion of test particles.

Particle uniformity is as critical to this method as to light scattering methods. Particles smaller than the reported diameter are more likely to be transmitted with both methods and to contribute an artifact within the analysis. They could significantly reduce the practical operating limit of the test. The primary limitation is not the detection sensitivity or accuracy of the fluorescence instrument, but is the quantity of smaller microspheres in the challenge material.

Since this test effectively eliminates size considerations from the analysis, the full spectrum of sizes may be analyzed. Insofar as the fluorescence spectrophotometer is concerned, there is no difference between a test solution using 0.03 µm particles or one using 3 µm particles. All that is analyzed is the freed dye in solution. This test is thus applicable to all membrane filters as well as more open filters using conventional fiber technology, including HEPA fiberglasses.

The method is not sensitive to miscellaneous contamination unless the contaminant fluoresces under similar conditions, which is unlikely. This means that debris from system components will not alter the overall results unless it is present in sufficient amounts to significantly increase the light- scattering properties of the sample. This technique offers a much improved performance test because fibers, membrane polymer fragments, and most extracted material from the filter and test system components present no fluorescent under normal conditions.

Unlike light scattering methods, the sensitivity is not dependent on the quality of the downstream dispersion, since the particles are dissolved before analysis. This may be especially valuable in testing hydrophobic or charged media where surfactants stabilizing the dispersion are stripped out of solution, causing agglomeration of the downstream particulates.

The choice of solvent is somewhat limited by the solvent's fluorescence. Water itself has a fluorescent spectral band, and methyl pyrrolidone has fluorescence about three times greater than water in the green region of the excitation spectrum, and more within the blue and UV regions. Since the background fluorescence is subtracted from the actual reading as a part of the testing procedure, the test data is still accurate, but the solvent's fluorescence limits the overall test sensitivity.

Direct Fluorescence Method

As shown in Figure 2, particles with diameters less than 100 nm (0.1 µm) have low light scattering properties, and can be read directly in fluorescence spectrophotometers. Although the scattered light present will somewhat alter the measured spectra from that of the pure dye, adequate fluorescent spectral shifts remain for good detection.

The direct fluorescence test is limited by the dispersion stability of the filtrate, but to a much lesser degree than the plain polystyrene light scattering test. This is because the fluorescent light signal is primarily dependent on the level of dye present in the sample and only secondarily upon attenuation as a function of light scattering. Under ideal conditions, this method can detect 100 nm suspended particles in the 1 part per billion concentration range, several orders of magnitude greater than the most sensitive light scattering methods. As with the dissolved method, the limitation to test accuracy is the number of smaller diameter fluorescent particles in the challenge material. Figure 4 shows the detection sensitivity of the direct and dissolved dye methods vs. light scattering methods.

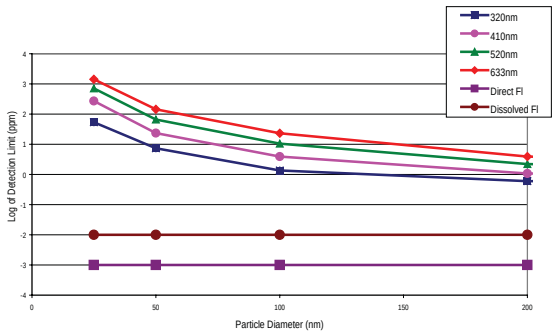

Figure 4. Light Scattering vs. Fluorescence: Approximate Threshold Detection Limits

7. SUMMARY AND CONCLUSIONS

Verification of membrane pore sizes for the filtration of aqueous liquids can be done with relatively modest outlays in equipment and training. Analysis of polystyrene latex challenge materials can be improved by using monodisperse polystyrene microspheres which have been prepared for use as filter challenge materials and particle size standards. New fluorescent particles have been described which make use of the physical retention properties of polystyrene spheres and the detection sensitivity of fluorescence spectrophotometers.

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