Designation: E 1343 – 90 (Reapproved 1997)ε1

Standard Test Method for Molecular Weight Cutoff Evaluation of Flat Sheet Ultrafiltration Membranes[1]

This standard is issued under the fixed designation E 1343; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

ε[1] NOTE—Section 11 was added editorially in April 1997.

1. Scope

1.1 This test method covers the evaluation of the molecular weight cutoff of flat sheet ultrafiltration membranes with cutoffs between 4500 and 1 000 000 daltons. The nonadsorbing characteristics of the test penetrant utilized by this test method permit the test to be performed on a wide variety of membrane substrates, excluding those which strongly adsorb dextran, from highly hydrophilic to highly hydrophobic. This test method is not applicable for microfiltration membranes with pore sizes of 0.01 µm or larger, nor for reverse osmosis or dialysis membranes with less than 4500 molecular weight cutoff. (It is possible that this test method could be modified to expand the range from 100 to 2 000 000 daltons.) This test method is not applicable to membrane materials that strongly adsorb dextrans since these materials will potentially change the value of the measured molecular weight cutoff and hence will invalidate the test results.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Summary of Test Method

2.1 The membrane is rinsed with purified water and installed in the test cell. The precalibrated dextran *T*-fraction feed is pumped through the cell and the flux through the membrane is set at the value of 0.0001 cm/s and an ultrafiltrate sample taken and compared to a sample of the feed for molecular weight distribution. Gel permeation chromatography is used to compare the feed to the permeate and the rejection at each 3-s data slice is calculated. The resulting rejection is then plotted as a function of the molecular mass average of the sample for the data slice.

3. Significance and Use

3.1 This test method provides a convenient, rapid, reproducible method of comparing the intrinsic properties of ultrafiltration membranes. The use of nonfouling dextrans allows a direct comparison of membranes without interference from materials that may foul one membrane and not affect another. The degree of correlation between this test and actual performance on a commercial feed stream has not been completely established; however, a membrane can be exposed to the fouling solution in question and then the effect of that foulant determined by then running the test and comparing to the results on an appropriate unfouled membrane. It should be made clear that this test method does not substitute for the actual testing of a commercial or experimental membrane on a feed stream of interest. The low transmembrane pressures, lack of adsorption of the test permeants onto the membrane, and low recovery/pass are intended to eliminate interferences such as polarization and fouling that mask the properties of the membrane. It is likely that any system operated in a commercial fashion will experience fouling, adsorption, and polarization to some degree as well as a "compaction" phenomenon over the first several h of operation.

4. Apparatus

4.1 The basic membrane test system consists of the following:

4.1.1 *Standard Flat Membrane, Stirred Ultrafiltration Test Cell*, for 62-mm membrane disc, modified as shown in Fig. 1,[2]

4.1.2 *Diaphragm Pump*, approximately 0 to 200 mL/min variable pumping range, 30 psig pressure capability at 100 mL/min, all wetted parts of 316 stainless steel and polyfluorocarbon construction,[3]

4.1.3 *Back Pressure Regulator and Pressure Gage*, 0 to 30 psig, 316 stainless steel,

4.1.4 *Electronic Digital Flowmeter*, with direct readout of volumetric flow in the approximate range 0.06 to 5 mL/min,

4.1.5 *Magnetic Stirplate with Tachometer*,[4]

[1] This test method is under the jurisdiction of ASTM Committee E-48 on Biotechnology and is the direct responsibility of Subcommittee E48.03 on Unit Processes and Their Control.

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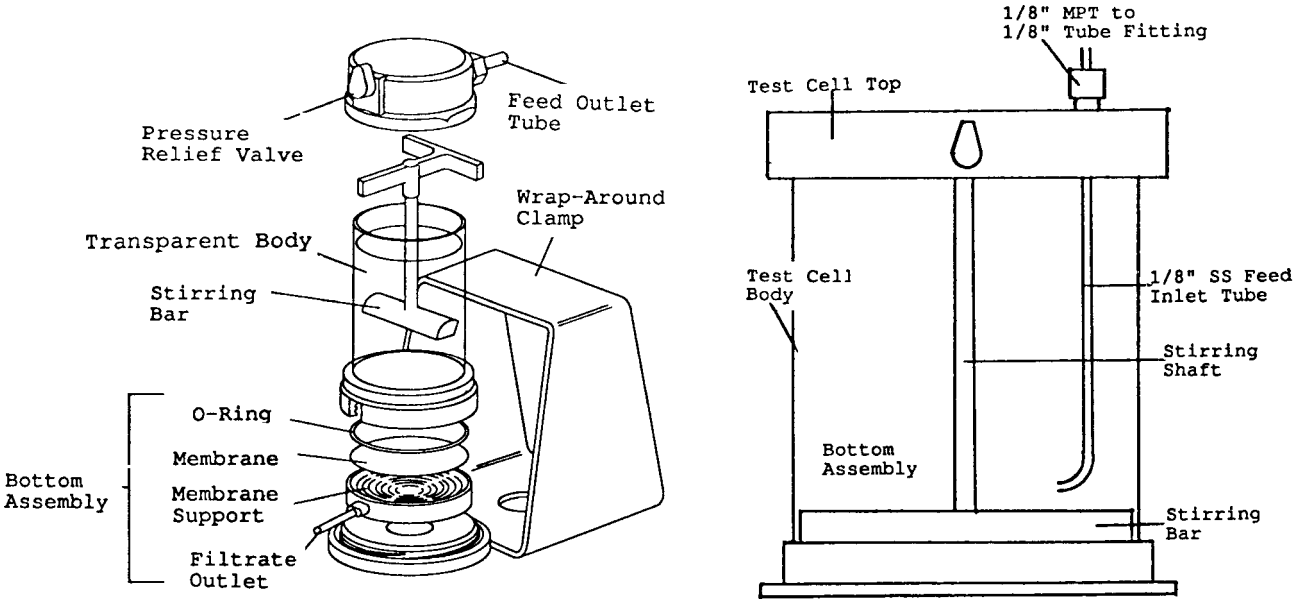
[2] Membrane equipment, model 8200 or equal, available from Amicon Division of W. R. Grace, 1114-T Avenue of the Americas, New York, NY 10036, has been found suitable for this purpose.

[3] Diaphram pump, model EP-C40 or equal, available from CHEM/TECH International Industries, 1655-T Des Peres P.O. Box 31000, St. Louis, MO 63131, has been found suitable for this purpose.

[4] Stirplate, model 4650-54, or its equivalent, available from Cole Parmer Spincadet, 7427-T N. Oak Park Ave., Chicago, IL 60648, has been found suitable for this purpose.



NOTE 1—The top of a standard stirred ultrafiltration cell was modified by drilling a hole through it and tapping it for ⅛-in. male pipe thread (MPT). A nylon fitting (⅛-in. tube to ⅛-in. MPT) was drilled through to allow the insertion of ⅛-in. tubing. The fitting was threaded into the top of the test cell. A stainless steel inlet tube was prepared as shown. The nylon fitting allows for the direction of flow to be at any height, and direction, or both. The existing fitting in the cap is used as an exit of flow. If the cell is equipped with a wrap-around clamp, it is modified to accommodate the extra fitting.

FIG. 1 Flow Through Modification of an Amicon-Stirred Cell

4.1.6 *Magnetic Stir Plate, Stirbar, and Container*, (1000 cm^3 Erlenmeyer) for test solution,

4.1.7 *Pulse Dampener*, 500-Ml, 316 stainless steel,

4.1.8 *Suitable Thermostatically Controlled Bath*, capable of 30 ± 0.2°C,

4.1.9 *Highly Reproducible and Stable Peristaltic Pump*, capable of flows in the 0.06 to 5 mL/min range,

4.1.10 *Appropriate Silicone Rubber Tubing*, for the peristaltic pump, and

4.1.11 *Inline Filters*, two, 2 and 15-µm, 316 stainless steel construction.

4.2 The gel chromatography system consists of the following:

4.2.1 *Good Quality High-Performance Liquid Chromatography (HPLC) Pump with Highly Reproducible Flow*,[5]

4.2.2 *HPLC Injection Valve*, with 20-mL sample loop and a position sensing switch,[6]

4.2.3 *Pair of Gel Permeation Columns*, capable of size exclusion separation of dextran polysaccharides in the 4500 to 10^6 molecular mass range,[7]

4.2.4 *Precolumn Filter to Protect the Columns (Guard Column)*,[8]

4.2.5 *Differential Refractometer HPLC Detector*, with 30°C water recirculating in the detector block and associated thermostatically controlled water source ±0.1°C,[9]

4.2.6 *Computing Integrator*, with data slicing and BASIC programming capabilities and suitable connecting cables for the integrator and detector. The integrator should include a printer capability to provide on-line output.[10]

5. Reagents and Materials

5.1 *Dextran T-fractions*—10 000 (*T*-10); 40 000 (*T*-40); 70 000 (*T*-70); and 500 000 (*T*-500) average molecular mass, supplied by the manufacturer with calibration curves of instantaneous and cumulative distribution versus molecular mass.

5.2 *Purified Water*—Treated with reverse osmosis, ion exchange and 0.2-µm filter to 18 MΩ quality.[11]

5.3 Sodium Azide.

6. Hazards

6.1 **Warning**—Sodium azide is toxic and flammable with a tolerance of 1 ppm in air, while the dextran *T*-fractions are nontoxic, the pressures utilized are hydraulic and are typically less than one atmosphere.

7. Preparation of Apparatus

7.1 *Assembly of Equipment—Chromatograph*:

[5] HPLC pump such as Beckman 110B Solvent Delivery Module, or its equivalent, available from Beckman Instruments, Inc., 2500-T Harbor Blvd., L-19 Fullerton, CA, has been found suitable for this purpose.

[6] Injection valve, such as 7010 HPLC, or its equivalent, available from Rheodyne Inc., 6815-T S. Santa Rosa Ave., P.O. Box 996, Cotati, CA 94928 has been found suitable for this purpose.

[7] Gel permeation columns such as Toyo-Soda G4000PW and G2000PW, with matching guard column or equivalents, have been found suitable for this purpose.

[8] Filters such as #84 560, or equivalents, available from Walters Inc., Mausner Equipment Co. Inc., 1304-T Prospect Ave. East Meadows, NY, have been found suitable for this purpose.

[9] Refractometer such as a R401 Differential, or its equivalent, available from Walters Inc., has been found suitable for this purpose.

[10] Computing integrator such as model SP.4270, or its equivalent, available from Spectra Physics, 5475-T Kellenberger Dr., Dayton, OH 45425, has been found suitable for this purpose.

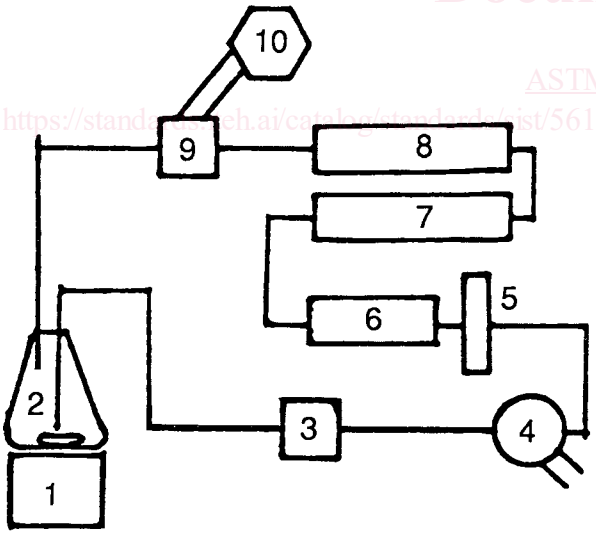
[11] System such as a Millipore Milli-Q, or its equivalent, available from Millipore Inc., 80 Ashby Rd., Bedford, MA, has been found suitable for this purpose.

7.1.1 The chromatograph should be assembled according to the manufacturer's instructions and following appropriate laboratory procedures. All tubing from the injector valve on is 0.010-in. internal diameter stainless steel 1⁄16-in. tubing and all connectors are zero dead volume fittings. All tubing ends should be deburred and polished prior to assembly. The liquid chromatographic columns are connected in series in the order: guard column and the two separation columns, in the sequence specified by the manufacturer. A sample of water with sodium azide is used in the reference cell. The signal from the detector is fed to the computing integrator that is set in data slice mode. The output from the integrator may be collected on a mainframe computer for further processing or it may be direct output using the printer system on the integrator, if wasted to drain. The complete arrangement of the chromatography system is shown in Fig. 2. The mobile phase is purified water with 0.05 % sodium azide.

7.2 *Assembly of Equipment—Test System*:

7.2.1 The test system is assembled according to the schematic shown in Fig. 3. The test cell and solution reservoir are immersed in the temperature controlled bath in container (see items 7, 2 and 12 in Fig. 3). All tubing on the high pressure loop (diaphragm pump, pulse dampener, in-line filters, test cell, back-pressure regulator) is ¼-in. stainless steel tubing, polyethylene tubing, or flexible braided stainless steel tubing. All other tubing is flexible plastic tubing such as PVC or silicone rubber tubing.

7.2.2 The test solution is composed of 0.25 wt % *T*-10, 0.10 wt % *T*-40, 0.10 wt % *T*-70, 0.20 wt % *T*-500, and 0.05 wt % sodium azide.



Identification of components:
(1) Magnetic stirrer,
(2) One-L mobile phase reservoir,
(3) Liquid chromatography pump,
(4) Sample valve with 20-mL sample loop and position sensing switch,
(5) Precolumn filter,
(6) Guard column,
(7) First liquid chromatographic separating column,
(8) Second liquid chromatographic separating column,
(9) Differential refractometer, and
(10) Constant temperature bath circulating 30°C-water through detector.

FIG. 2 Liquid Chromatography Apparatus for Molecular Weight Cutoff Determinations

7.3 *Assembly of Equipment—Pretest*:

7.3.1 All pressurized systems should be pressure tested at their operating pressures before the membrane testing procedure is initiated.

7.3.2 The chromatography system should be run at 1.5± 0.1 cm^3/min for 1 h to check for leaks in the system. All leaks should be corrected by either retightening the fittings that are leaking or by replacing the fittings.

7.3.3 The membrane test system should be run at 200 ± 5 cm^3/min for 1 h with a nonpermeable disk of plastic film in the test cell. All leaks should be repaired and an estimate of the pressure/flow stability of the system obtained.

8. Procedure

8.1 *Calibration Curve*:

8.1.1 Load the program into the computing integrator. A typical program is shown in Table 1.

8.1.2 Set the parameters on the integrator to appropriate values determined by the *T*-fraction chromatograms in accordance with the instructions with the integrator.

8.1.3 Set the pump rate at 0.80 cm^3/min, the detector thermostat to 25°C, and allow the system to equilibrate for 1 h. (Systems can be allowed to run continuously and the pump idled at 0.1 c^3/min when not in use.)

8.1.4 Inject a sample of each of the four dextran *T*-fractions to generate a curve of elution time versus molecular mass. The standard calibration curves supplied by the manufacturer may be used to convert elution fraction to molecular mass. To avoid errors due to a small portion of the *T*-10 eluting after the column volume, only the integrated curve of each fraction up to the peak was used for the calibration. By generating a cumulative curve from the *T*-fraction data but assuming that the peak is 100 % we can then obtain a calibration curve such as that shown in Fig. 4. A3-term fit for the standard curve was obtained with 0.998 correlation. Generally accuracy is much better if elution mass is used rather than elution time for the molecular weight determination. This correction can be easily made by collecting the eluent from the detector for the time period of the run and weighing it. This corrects for minor fluctuations in the pump rate.

8.2 *Test Procedure*:

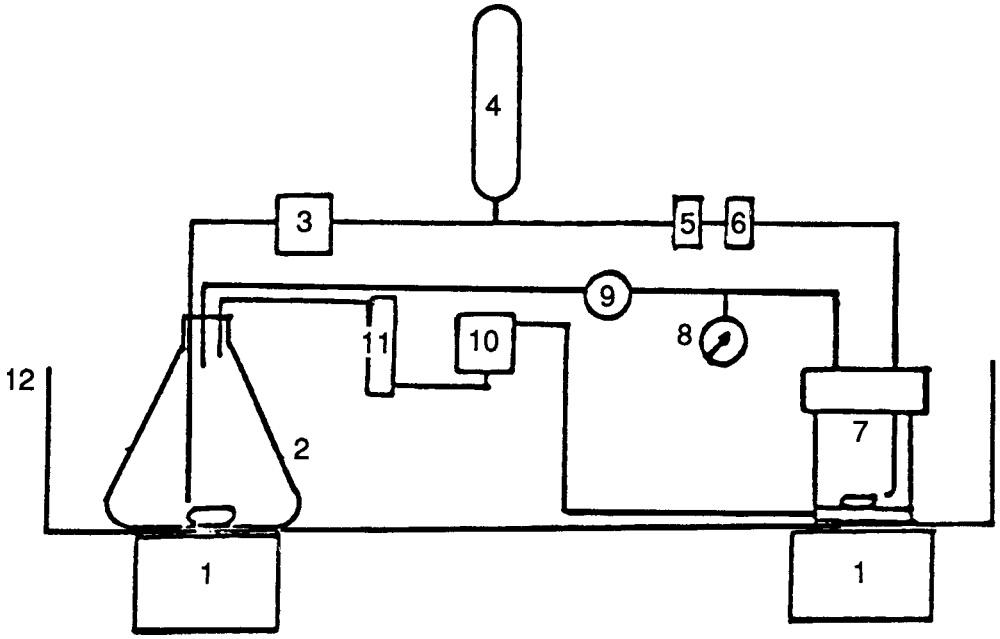
8.2.1 Although it is not necessary to verify beforehand that the membrane to be tested is of good integrity, it is helpful to challenge the membrane in a test cell with 0.2 wt % blue dextran 2000 at 10 to 30 psig. A blue filtrate indicates that the molecular weight cutoff of the sample is greater than 2 000 000 daltons, outside of the range of the test. This membrane sample must not be used for the subsequent molecular weight cutoff evaluation as it will be contaminated with the blue dextran and hence may give a spurious value in this test.

8.2.2 Start the constant temperature bath, and all components of the chromatograph (if not already running).

8.2.3 Allow the chromatograph to equilibrate for 1 h.

8.2.4 While the chromatograph is equilibrating, select the membrane sample, rinse with distilled water, and insert the membrane into the test cell.

8.2.5 Assemble the test cell and connect to the test apparatus via the feed, permeate, and effluent tubes.



Identification of components:
(1) Magnetic stirrers,
(2) Erlenmeyer flask used for test solution reservoir,
(3) Diaphragm pump,
(4) Five-hundred c^3 sample cylinder used as a pulse dampener,
(5) Fifteen-µm in-line filter,
(6) Two-µm in-line filter,
(7) Ultrafiltration cell modified for flow through of test solution,
(8) Zero to 15 psig (or other suitable range) pressure gage,
(9) Two to 30 psig (or other suitable range) back pressure regulator,
(10) Highly reproducible peristaltic pump,
(11) Flowmeter, and
(12) Constant temperature bath.

NOTE 1—The magnetic stirrer under the cell may be equipped with a tachometer to monitor the stirring rate.

FIG. 3 Test Apparatus for Molecular Weight Cutoff Determinations

8.2.6 Open the back pressure regulator so that no pressure is on the system.

8.2.7 Check the level of the dextran *T*-fraction solution and add solution if less than 750 mL is in the reservoir.

8.2.8 Start the diaphragm pump and adjust to a flow of 100 ± 10 cm^3/min.

8.2.9 Start the magnetic stirrer under the test cell and adjust to 250 ± 25 r/min.

8.2.10 Close the back pressure regulator to a pressure of 6 ± 0.5 psi on the gage.

8.2.11 Start the peristaltic pump and adjust to a flow rate of 0.17 ± 0.01 c^3/min as indicated on the flowmeter (0.17 cm^3/min corresponds to a transmembrane velocity of 0.0001 cm/s through the membrane and is intended to be too low for polarization effects to be important). The membrane test area is a function of the test cell used. Refer to the manufacturer's literature for the exposed area of membrane.

8.2.12 Wait ½ h for the test apparatus to equilibrate.

8.2.13 Take a 5-cm^3 sample of the ultrafiltrate and a 5-cm^3 sample of the test dextran solution. Sample the ultrafiltrate by disconnecting the feed line between the peristaltic pump and the flowmeter. Repeat after an additional ½ h has elapsed.

8.2.14 *Optional*—A second set of samples at a peristaltic pump flow rate of 0.34 ± 0.01 c^3/min may be taken. (This can be used to check for concentration polarization effects.)

8.2.15 Inject the samples into the chromatograph with the test solution injected first, followed by the sample. During the test solution run collect the eluent and determine the flowrate gravimetrically to ±0.001 mL/min. Collection for 10 min in a tared container and weighing on a milligram balance is sufficiently accurate. Any reduction in flowrate >0.005 mL/min may indicate failure of the pump seals or an increase in column back pressure. Check both the pump seals and column pressure before proceeding.

9. Calculations

9.1 When the integrator has printed all the "raw" data slice areas for the test sample, run the program indicating that this is a test solution. After all queries are answered, the computer will store the slice areas into an array for future use. After each filtration sample is finished and all the "raw" data have been printed, run the program again indicating that this is a sample of the filtrate. The computer will then generate a table of molecular mass versus rejection using the following equation:

$$\%\,R = (1 - Cf/Ct)100$$

where:
$\%\,R$ = percent rejection,
Cf = dextran concentration in filtrate, and
Ct = dextran concentration in test solution for the molecular mass of interest.

9.2 This data can be plotted onto a log molecular weight versus rejection to generate a complete sieving curve. A second curve is generated from the duplicate samples taken (as described in 8.2.13). Additional curves are also plotted from optional samples (8.2.14). From this and the molecular weight