Removal of Endotoxin from Water by Microfiltration through a Microporous Polyethylene Hollow-Fiber Membrane

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The microporous polyethylene hollow-fiber membrane has a unique microfibril structure throughout its depth and has been found to possess the functions of filtration and adsorption of endotoxin in water. The membrane has a maximum pore diameter of approximately 0.04 μm, a diameter which is within the range of microfiltration. Approximately 10 and 20% of the endotoxin in tap water and subterranean water, respectively, was smaller than 0.025 μm. Endotoxin in these water sources was efficiently removed by the microporous polyethylene hollow-fiber membrane. Escherichia coli O113 culture broth contained 26.4% of endotoxin smaller than 0.025 μm which was also removed. Endotoxin was leaked into the filtrate only when endotoxin samples were successively passed through the membrane. These results indicate that endotoxin smaller than the pore size of the membrane was adsorbed and then leaked into the filtrate because of a reduction in binding sites. Dissociation of H-labeled endotoxin from the membrane was performed, resulting in the removal of endotoxin associated with the membrane by alcoholic alkali at 78% efficiency.

The removal of endotoxin (lipopolysaccharide [LPS]), a major pyrogen, from water or parenteral substances is of major importance for manufacturing processes and in quality control. Although many methods (4, 8, 10, 14–19, 21, 29, 31, 32; N. S. Harris and R. Feinstein, U.S. patent 3,944,391, March 1976) for removing endotoxin have been used, ideal and reliable methods are still needed. The recent progress of membrane technology led to an examination of the potential use of molecular filtration membranes such as those used in reverse osmosis (18) or ultrafiltration (3, 20, 29, 32) for water samples. However, these methods encountered the difficulty of low water flow rates.

The LPS monomer is an amphiphile with a large hydrophilic polysaccharide chain and a hydrophobic fatty acid tail. The LPS monomer can be found in various aggregate forms (7, 35). LPS in large forms can be converted into smaller forms under various conditions (13, 22, 27). The converted forms may then be conjugated to other compounds to form hybrids (23, 26). Such aggregation and disaggregation of LPS brings about bewildering and inconsistent results in the removal of endotoxin by filtration. Therefore, we focused on the removal of endotoxin smaller than the pore size of the membrane by adsorption.

The microporous polyethylene hollow fiber (EHF; Mitsubishi Rayon Co., Tokyo, Japan) (M. Shindo, T. Yamamoto, O. Fukunaga, and H. Yamamori, U.S. patent 4,401,567, August 1983) was developed into a water purification device (11) which was found to remove various bacterial cells as well as authentic LPS (Enterobacter clostridii O111:B4; Difco Laboratories, Detroit, Mich.) from water. In addition, it was previously found that an adsorbent prepared from this EHF had the ability to adsorb LPS in water (12). Therefore, the EHF membrane appears to utilize a combination of adsorption and filtration of endotoxin in water when used as a filter membrane. To clarify the mechanisms of endotoxin removal, we examined the contributions of the functions of adsorption and filtration of endotoxin. This was done by examining the following: (i) the maximum pore size of the EHF membrane; (ii) the removal of endotoxin from various water samples by the EHF membrane; (iii) the results of an analysis of the size distribution of endotoxin activity; and (iv) the results of the dissociation of H-labeled LPS from the EHF membrane.

MATERIALS AND METHODS

EHF and module. The EHF was produced from high-density polyethylene as described previously (Shindo et al., patent 4,401,567, August 1983). Figure 1 shows a scanning electron micrograph (JSM 35C electron microscope; Nihon Denshi Co., Tokyo, Japan) of the inner surface of the EHF membrane (code EHF 390 C). The outer surface was virtually the same (data not shown). The EHF membrane has good mechanical strength and chemical stability and does not contain any fillers or plasticizers. Its specifications are as follows: outer diameter, 390 μm; membrane wall thickness, 55 μm; porosity, 63% (34); bubble point, 4.85 ± 0.13 kg/cm² in 99% ethanol (1); water flux, 3.6 liters/min per m² of membrane surface area at a pressure of 0.5 kg/cm² at 25°C; and nitrogen-adsorbing surface area, 31.7 m²/g (2). A module was formed with 128 filaments of the EHF membrane, a polycarbonate jacket, and a potting agent of polyurethane (Nippon Polyurethane Co., Tokyo, Japan) (Fig. 2). The total outer surface area of the EHF membrane was approximately 70 cm²/116 mg of the module. The module was confirmed to have no sealing insufficiency by feeding water at a pressure of 1 kg/cm² for 1 min. Scaling was confirmed to be sufficient unless water leaked through. To make the module permeable to water, 30 ml of 99% ethanol was passed through the membrane, which was then rinsed with 50 ml of nonpyrogenic water (Otsuka Pharmaceutical Co., Naruto, Japan). The prewashed module was used without being dried.

Pore characteristics of the EHF membrane. A suspension of beads in 40 ml of water (0.1% [wt/vol]) was applied to the EHF membrane in the module with a Mini-Pump (TMP 10H;
Toyo Kagaku Sangyo Co., Tokyo, Japan) at a flow rate of 8 ml/min (Fig. 2). Air in the module was vented out through two air outlets. A 2-ml fraction was collected. The maximum concentration of the beads eluted in the filtrate was interpreted as a rejection of the EHF membrane and expressed as a percentage of the total concentration. Each value represented the mean of three or four determinations. Uniform beads, colloidal silica, and polystyrene in monodispersed form were obtained from Maruwa Bussan Co., Tokyo, Japan (originally from E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.), Dow Chemical Co., Indianapolis, Ind., and Shokubai Chemical Co., Tokyo, Japan, respectively. For assessment of the concentration of beads being used, the A210 to A280 (different wavelengths were used depending on the type and size of the beads) was measured with a Shimadzu UV-250 spectrophotometer attached to an OPI-2 recording unit (Kyoto, Japan). A standard concentration curve was prepared by diluting the beads with distilled water.

Treatment of water samples. Water samples obtained in Japan were immediately used either for determination of endotoxin activity or for filtration treatment. A sample from Philadelphia, Pa., was frozen and airmailed. The water flux in the module was adjusted to 0.5 ml/min at 25°C. The filtrate outlet of the module was wiped with cotton saturated in 70% ethanol before sample collection.

Analysis of size distribution of endotoxin activity. Various filter membranes with nominal pores were purchased from Millipore Corp., Bedford, Mass., and Nuclepore Corp., Pleasanton, Calif. The membranes were extensively washed before use with nonpyrogenic water, and water drops on the membrane surface were removed by shaking the membrane. The membrane was set in an adaptor for an injection syringe. The water sample in the syringe was forced to pass through the membrane. The first drops were discarded. A 2-ml filtrate sample was collected, and the endotoxin activity was determined. Preheating of the water sample was done in a water bath for 30 min.

Determination of endotoxin activity. The Limulus amoebocyte lysate-based assay kits Pyrodict (assay A) and Toxicolor (assay B), purchased from Seikagaku Kogyo Co., Tokyo, Japan, were used. These assays have been described previously (5, 6, 9). Incubation was done at 37°C for 30 min. The wavelengths were read at 405 nm for assay A and at 545 nm for assay B. All data represent the mean of duplicate determinations and were expressed in amounts equivalent to purified LPS of E. coli O111:B4 (36). All glassware was depyrogenated by being heated at 250°C for 2 h. Nonpyrogenic water was used both for the dilution of water samples, if necessary, and for the controls.

Pyrogen tests on rabbits. Pyrogen tests were carried out in accordance with the Japan Pharmacopeia X. Three rabbits were used for each sample, and their body temperatures were measured at 1 h after intravenous injection. Concentration of the endotoxin in tap water was done with a rotary evaporator in vacuo at 30 to 35°C.

Column chromatography with Sephadex G-75. A column (1.6 by 20 cm) of Sephadex G-75 (Pharmacia Japan, Tokyo) was washed extensively with nonpyrogenic water. The flow rate was adjusted to 1 ml/min. A 2-ml fraction was collected.

Bacterial strains and their cultivation. E. coli and Salmonella minnesota were from our stock and were grown in...
FIG. 3. Plot of rejection versus particle size for the EHF membrane.

Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 18 h. For determination of viable cells, the cultures were diluted with sterilized physiological saline (Otsuka Pharmaceutical Co.). Viable cells were counted in heart infusion agar (Eiken Kagaku Co., Tokyo, Japan) after cultivation at 37°C for 48 h. All values represent the mean of triplicate determinations.

Preparation of [3H]LPS. [3H]LPS was prepared from the LPS isolated from S. minnesota R595 by 3H-labeled gas exposure (New England Nuclear Corp., Boston, Mass.) (30). The specific radioactivity was 1.14 mCi/mg (3.0 mCi/ml of water). A mixture of cold LPS (1.0 mg) and [3H]LPS solution (30 μl) in water (10 ml) was dispersed with a cell disruptor (Sonifier cell disruptor 200; Branson Sonic Power Co., Danbury, Conn.) at 20 kc and 20 W for 3 min on an ice bath. The sonicated solution (2-ml aliquots) was applied to a column of Sephadex G-75. The column was eluted with nonpyrogenic water. A 2-ml fraction was collected. The 3H in 100-μl samples of the filtrate was measured with a counter (1215 Rackbeta; LKB-Wallac, Turku, Finland) and an aqueous counting scintillant (ACS II; Amersham Corp., Arlington Heights, Ill.). Fractions 6 and 7 were combined, LPS (76%) was recovered by measuring the A_{280}, and the [3H]LPS (26%) was recovered from the total 3H. Radioactivity in the latter fractions from the column was discarded. The specific radioactivity of the [3H]LPS was 11.84 μCi/mg.

Preparation of the 3H-labeled EHF membrane. The purified [3H]LPS solutions were combined, and 10 ml of this solution was loaded onto the EHF membrane in the module and subsequently washed with distilled water (10 ml) at a flow rate of 1 ml/min. The module was broken, and the EHF membrane was removed for further study.

Dissociation of 3H from the [3H]LPS-adsorbed EHF membrane. The [3H]LPS-adsorbed EHF membrane was cut into 1-cm-long pieces. The cut fiber pieces (60 mg) were suspended in 10 ml of solvent and gently stirred at 35°C. A 200-μl sample of the solvent was removed, and the 3H was measured. The 3H remaining in the fibers after 120 min was also determined by measuring the whole fiber pieces.

RESULTS

Maximum pore size of the EHF membrane. A determination of the maximum pore size of the EHF membrane is crucial in perceiving the removal of contaminant materials by filtration. Because the EHF membrane used for the module (Fig. 2) has lattice-like pores on its surfaces (Fig. 1)

TABLE 1. Endotoxin activity in various water samples before and after treatment with the EHF membrane

<table>
<thead>
<tr>
<th>Water</th>
<th>Location</th>
<th>Endotoxin concn (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before filtration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>assay A</td>
</tr>
<tr>
<td>Tap</td>
<td>Tokyo, Japan</td>
<td>1.98</td>
</tr>
<tr>
<td>Tap</td>
<td>Osaka, Japan</td>
<td>9.00</td>
</tr>
<tr>
<td>Tap</td>
<td>Nagoya, Japan</td>
<td>5.20</td>
</tr>
<tr>
<td>Tap</td>
<td>Philadelphia, Pa.</td>
<td>7.29</td>
</tr>
<tr>
<td>Subterranean</td>
<td>Nagoya, Japan</td>
<td>2.19</td>
</tr>
<tr>
<td>Distilled</td>
<td></td>
<td>0.30</td>
</tr>
</tbody>
</table>

* Sampling and assaying were performed in the summer of 1983.  
* A 50-ml filtrate was collected, and endotoxin activity was assayed.  
* Freshly distilled water made in a glass apparatus was stored in a plastic bottle for a few days at room temperature.
and a tortuous fiber maze throughout its depth, like the Celgard membrane (24), the pore size was studied. Filtration of spherical particles of a uniform diameter has been well studied (25, 28). The EHF membrane was estimated to have a maximum pore size (100% rejection by extrapolation) of approximately 0.04 μm (Fig. 3). Therefore, the maximum pore size of the EHF membrane is of microfiltration grade, i.e., a pore size ranging from 0.02 to 10 μm (20).

Removal of endotoxin from various water samples. Municipal tap water contains a mixture of various sources of endotoxin. Although tap water samples in both Japan and the United States contain nanogram levels of endotoxin activity, the filtrate from the EHF membrane had an activity of less than 10 pg/ml, as determined by assay A. To determine low levels of endotoxin activity, we used a modified assay system, assay B. Because p-nitroaniline hydrolyzed from a substrate is converted to a red diazo derivative, assay B is 10-fold more sensitive than assay A. All filtrates contained endotoxin activity at 3 to 4 pg/ml. Even subterranean water and single-distilled water for laboratory use contained high endotoxin activity. Filtrates of these water samples contained endotoxin activity at 3 pg/ml (Table 1).

Elimination of factors in water filtrates which affect the endotoxin assay system. To eliminate possible contamination factors in filtrates which affect the endotoxin assay system, a filtrate from the module containing nonpyrogenic water was added to various concentrations of a reference LPS (E. coli O111:B4). Virtually no effect was observed on the standard curve of the reference LPS in assay A for the filtrate (data not shown).

Analysis of the size distribution of endotoxin activity in two
TABLE 2. Concentrations of endotoxin in filtrates from tap and subterranean water

<table>
<thead>
<tr>
<th>Filtrate vol (liters)</th>
<th>Endotoxin (ng/ml)* in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tap water</td>
</tr>
<tr>
<td>Unfiltered sample</td>
<td>3.57</td>
</tr>
<tr>
<td>0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.02</td>
</tr>
<tr>
<td>10</td>
<td>0.08</td>
</tr>
<tr>
<td>13</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* The total organic concentrations in tap water and subterranean water were 1.7 and <0.1 mg/liter, respectively.

**water sources.** Tap water from Nagoya City was filtered through various membranes with nominal pore sizes at several temperatures. Four filtrates at various temperatures had similar profiles of size distribution. Endotoxin smaller than 0.025 μm (designated here as small-sized endotoxin) represented approximately 10% of the total (Fig. 4A). Another sample, subterranean water from Nagoya City, had a profile of size distribution at 15°C similar to that of the tap water, with a small-sized endotoxin concentration of approximately 20% (Fig. 4B).

**Removal of the endotoxin activity from two water sources.** To remove the endotoxin activity from the tap water and subterranean water samples, 13 liters of water from each source was passed through each EHF membrane module, and the endotoxin activity in the filtrates was determined. For tap water, the endotoxin activity appeared gradually in the last few fractions. For subterranean water, almost all of the endotoxin activity was removed from 13 liters (Table 2).

**Size of endotoxin in tap water samples.** The endotoxin activity in tap water was analyzed by column chromatography with Sephadex G-75. Two peaks appeared, with a total recovery of 86% (Fig. 5A). The first peak was at 68% of the eluate. Analysis of the size distribution of both peaks was performed by using a membrane with a nominal pore size of 0.025 μm. Approximately 5% of the endotoxin of the first peak and approximately 95% of that of the second peak passed through the membrane. In addition, the endotoxin activity in the 13-liter tap water filtrate (Table 2) was analyzed. The peak fraction of activity coincided with the second peak fraction of activity in tap water (Fig. 5B).

The 13-liter tap water filtrate (Table 2) was filtered through various membranes with different nominal pore sizes, and approximately 90% of the endotoxin in the filtrate was found to be smaller than 0.05 μm (data not shown).

**Pyrogen tests on rabbits.** To determine whether the endotoxin activity in tap water causes a rise in body temperature, pyrogen tests on rabbits were carried out. To obtain a high concentration of pyrogen activity, a tap water sample containing low levels of endotoxin activity was chosen. The tap water of Nagoya City (endotoxin activity, 0.6 ng/ml) was first evaporated to about 1/70th its original volume. The concentrated sample had an endotoxin activity of 43.4 ng/ml. As a result, the concentrations of and increases in endotoxin activity were in good correlation. A part of the concentrated

![FIG. 5. Chromatographic profile of endotoxin activity in tap water. (A) Tap water (endotoxin activity, 2.4 ng/2 ml) analyzed on a Sephadex G-75 column. (B) Analysis of the fractions corresponding to 0.1-liter filtrates (×) and 13-liter filtrates (○) in Table 2.](http://aem.asm.org/Downloaded from http://aem.asm.org)
sample was then treated with the EHF membrane. The filtrate contained an endotoxin activity of less than 10 pg/ml. Although the tap water showed no pyrogen activity in rabbits, the concentrated endotoxin caused an increase in body temperature of 1.5°C at 2 h after intravenous injection. However, filtration of the concentrated endotoxin eliminated all the pyrogenic response from the rabbits. In a parallel experiment, reference LPS from *E. coli* O111:B4 at 56 ng/ml raised the body temperature by 1.65°C (Fig. 6). Thus, endotoxin activity in a tap water sample and increased temperature showed a good correlation.

**Simultaneous removal of cultured bacterial cells and their endotoxin activity.** Gram-negative bacteria produce LPS on their cell surfaces and liberate it into the surrounding medium under certain environmental conditions. To remove the bacterial cells and LPS, two strains of *E. coli* and one strain of *S. minnesota* were grown. Diluted cultures were filtered through the EHF membrane module. All filtrates contained no viable cells and had undetectable endotoxin activity (Table 3). A profile of the endotoxin activity in the diluted cultures was obtained by column chromatography with Sephadex G-75. Three diluted cultures showed only a peak corresponding to the first peak shown in Fig. 5A; no peak

**TABLE 3.** Simultaneous removal of cultured bacterial cells and their endotoxin activity

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>No. of viable cells per ml (10^6)</th>
<th>Endotoxin concn (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before filtration</td>
<td>After filtration</td>
</tr>
<tr>
<td><em>E. coli</em> O113</td>
<td>3.0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. coli</em> O111:B4</td>
<td>4.5</td>
<td>0</td>
</tr>
<tr>
<td><em>S. minnesota</em> R595</td>
<td>2.0</td>
<td>0</td>
</tr>
</tbody>
</table>

* A 50-ml filtrate was collected, and endotoxin activity and viable cells were determined.
corresponding to the second peak was observed (data not shown).

Next, all cultures were filtered through membranes with a pore size of 0.025 μm. The filtrate from the E. coli O113 culture contained the smallest endotoxin activity (26.4%), whereas the filtrates from the E. coli O111:B4 and S. minnesota R595 cultures contained only 0.2 and 1.0% of the small-sized endotoxin activity, respectively (Table 4).

Adsorption and dissociation of the [3H]LPS. To determine the dissociation of endotoxin from the EHF membrane, [3H]LPS prepared from LPS isolated from S. minnesota R595 was passed through the EHF membrane. An attempt was made to regenerate the [3H]LPS-adsorbed membrane by rinsing it with certain solvents. Water as a solvent dissociated 5.6% of the [3H] Ethanol at a 70% concentration removed 43% of the [3H] from the EHF membrane, and ethanolic alkali removed 78% of the [3H] (Table 5).

**DISCUSSION**

Most filter membranes have been designed to remove contaminant materials by having a pore size that prevents the contaminant materials from flowing through the membrane. The removal efficiency and the accuracy of the filter membrane are directly influenced by the following: (i) the membrane structure, e.g., a top skin layer and a porous sublayer; (ii) the water quality; (iii) the flow rate; and (iv) other variables. The EHF membrane has lattice-like pores on its surface which form a tortuous fiber maze throughout the depth of the membrane. This structure is similar to that of Celgard membrane, which is made from polypropylene (24). Although the pore size of the EHF membrane seems large (Fig. 1), the membrane has been experimentally found to have a maximum pore size of 0.04 μm. Therefore, the EHF membrane can remove contaminant materials larger than 0.04 μm by the filtration process. The endotoxin activity in tap water was analyzed by column chromatography with Sephadex G-75 and showed two peaks. The proportions of the activity in the first peak and the second peak were 68 and 32%, respectively (Fig. 5A). Approximately 95% of the second peak contained endotoxin smaller than 0.025 μm. The small-sized endotoxin corresponding to the second peak was experimentally found to be removed from water samples by the EHF membrane (Table 2). Furthermore, endotoxin activity gradually appeared in the filtrates after the successive feeding of endotoxin, indicating that small-sized endotoxin passed through the membrane because of a reduction in adsorption sites. We have already demonstrated the adsorption characteristics of EHF for endotoxin and tetrachloroethylene (12). The organic compounds adsorbed were, in turn, well dissociated from the membrane after an ethanol rinse (11). To evaluate the dissociation of endotoxin from the EHF membrane, [3H]LPS was used. Alkali is known to destroy the endotoxin molecule (15). Ethanol releases the hydrophobic interaction. Therefore, alcoholic alkali is an ideal solvent for regeneration of the EHF membrane. During use of the EHF membrane for tap water, rinsing of the membrane with 70% ethanol-0.1 N NaOH caused rejuvenation of the membrane. As for water quality, not only the concentration of endotoxin but also the concentrations of other organic compounds in water have an effect. The efficiency of removal of endotoxin may be affected because other organic compounds can be adsorbed on the same binding sites as is endotoxin. The total concentrations of organic compounds in tap water and subterranean water were 1.7 mg/liter and less than 0.1 mg/liter, respectively. The greater efficiency in removing endotoxin from subterranean water seemed to be attributable to the quantity of the contaminated solids (Table 2). The hydrophobic compounds were more efficiently bound to the membrane (unpublished data). Further comparative adsorption characteristics are currently under way. Since the adsorbing capacity of an adsorbent for endotoxin made from this EHF resulted in an insignificant difference at a pH range of 5.1 to 9.1 (unpublished data), the effect of pH on adsorption in the filtration process may not exist in this pH range.

A confident analysis of endotoxin activity is of utmost importance in solving the problems associated with endotoxin. Based on the clarification of the biochemical mechanism of gelation of Limulus amoebocyte lysate, Iwanaga and his collaborators (9) recently developed a sensitive and quantitative method for assaying endotoxin. This method, the synthetic chromogenic substrate method, is recommended for its potential application to water, parenteral substances, and blood samples. It must be noted that this assay system only detects the active forms of endotoxin (not the amount of it) and other reactive substances, such as β-glucan and its analogs. Recently, various water samples were tested by using this assay system, and the reactive substance in tap water samples was interpreted to be endotoxin (33, 37). Because the EHF membrane is hydrophobic, it has been found to possess interesting characteristics, e.g., the adsorption of organic compounds, including endotoxin. The EHF membrane has good mechanical strength in addition to some advantages of hollow-fiber-type filters over other membrane configurations (32). Therefore, the EHF membrane can be used to remove endotoxin from water used in laboratories.

**TABLE 4. Size distribution of endotoxin activity in cultures**

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Endotoxin activity (ng/ml) in:</th>
<th>Diluted culture</th>
<th>Filtrate (from 0.025-μm membrane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli O113</td>
<td>252.2</td>
<td>66.7</td>
<td></td>
</tr>
<tr>
<td>E. coli O111:B4</td>
<td>309.8</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>S. minnesota R595</td>
<td>20.3</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

* A bacterial cell was centrifuged (1,000 × g, 10 min), and the supernatant was removed. The supernatant was dialyzed 10 times with nonpyrogenic water. Endotoxin activity in either diluted cultures or filtrates was determined by assay A.

**TABLE 5. Dissociation of [3H] from the [3H]LPS-adsorbed EHF membrane**

<table>
<thead>
<tr>
<th>Rinse agent</th>
<th>Duration of treatment (min)</th>
<th>Radioactivity (cpm) in:</th>
<th>Total radioactivity (cpm)</th>
<th>% Dissociation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0</td>
<td>2.0 × 10^3</td>
<td>8.9 × 10^3</td>
<td>5.6</td>
</tr>
<tr>
<td>Ethanol</td>
<td>60</td>
<td>2.9 × 10^3</td>
<td>7.2 × 10^3</td>
<td>43</td>
</tr>
<tr>
<td>Ethanol (70%)</td>
<td>60</td>
<td>5.5 × 10^3</td>
<td>1.37 × 10^4</td>
<td>78</td>
</tr>
<tr>
<td>NaOH (0.1 N)</td>
<td>120</td>
<td>6.0 × 10^3</td>
<td>7.7 × 10^3</td>
<td>104</td>
</tr>
</tbody>
</table>

* Rinsing agent: Ethanol (70%), NaOH (0.1 N).
pharmaceutical houses, and certain health care environments such as dialysis centers.

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LITERATURE CITED


