

# FEASIBILITY OF CERAMIC ULTRA- AND NANOFILTRATION MEMBRANES FOR REMOVAL OF ENDOTOXINS

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## ABSTRACT

The removal of endotoxins, a potential contaminant of dialysis water and dialysate, is a very difficult task. The endotoxin removal capacity of commercial ceramic membranes with a nominal molecular weight cut-off of < 1,000 and adsorber membranes was investigated. The dead-end filtration results showed that all investigated ceramic membranes produce water meeting the European standards when challenged with low endotoxin concentrations, but only one membrane type succeeded at high endotoxin concentrations. In addition, we present preliminary analysis of the factors determining bacterial fragment removal from water of different ceramic and polymeric adsorptive membranes.

## KEYWORDS

ceramic membrane, charged membrane, retention efficiencies, dialysis, water treatment

## 1. Introduction

The quality of water in dialysis fluid varies considerably and as a result, dialysate is often contaminated by large amounts of bacteria and endotoxins. The latter may cross the dialysis membrane and so endotoxins are a potential contaminant of dialysis water and dialysate. Endotoxins are lipopolysaccharides (LPS) derived from the cell membranes of Gram-negative bacteria and are responsible for organization and stability of cell membrane. The molecular weight of endotoxin is about 10 kDa [1]. They can indirectly cause fever because they stimulate synthesis in host cells and release endogenous proteins. Membrane properties and operating pressures can cause the bacteriological potential to favor passage of endotoxin fragments from the dialysate into the blood side and to trigger monocyte activation and cytokine production during high-flux dialysis with bicarbonate and during membrane re-use. For these reasons, a sterile dialysate free of endotoxins and other cytokine inducing factors will likely become a standard. Ultrafiltration across membranes made of polysulphone, polyamide or polyethylene has been shown to remove bacteria, endotoxins and their fragments and other cytokine inducing factors from water by a combined effect of filtration and adsorption. Also, the removal of bacterial endotoxins from membrane adsorbers was shown [2]. However, use of these modules is limited by the operation time which is dependent upon polymer type and device. Repeated

cleansing with aggressive agents may damage the membrane and may change the performance of the filter [3, 4].

Ceramic membranes are generally more resistant to harsh operating conditions (extremes of pH, high temperatures and pressure etc.) than polymeric membranes, and may represent an alternative for endotoxin removal. They can be re-used many times and are easily regenerated. Membranes will be used to reduce endotoxin content of product streams in the downstream processing of biopharmaceutical products. Additionally, ceramic membrane ultrafiltration has been validated for the production of water meeting the requirements of pyrogen-free water for injection standards [5]. This ability can be put to immediate use in other pharmaceutical water systems that require pyrogen removal but are not limited by USP definitions of production methods—bulk active pharmaceutical production is one example.

In this paper, the adsorption capacities of different membranes for bacterial fragments such as LPS are investigated. Additionally, we present results for the capacity of different membranes to retain endotoxins from *E. coli* (Serotype 055:B5) in long-term runs.

## 2. Materials and Methods

### Membranes

Different types of commercial tubular ceramic membranes for ultra- and nanofiltration (Table 1, Fig. 1) and different types of commercial adsorber membranes (Tab.2, Fig. 2) were investigated for endotoxin removal.

**Table 1:** Properties of the ceramic membranes used in this investigation

Ceramic Membranes			
Identity	Membrane #1	Membrane #2	Membrane #3
Geometry	Single channel	Single channel	Multiple channels
Number of channels	1	1	3
Material	Al <sub>2</sub> O <sub>3</sub> /TiO <sub>2</sub> /ZrO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub> /TiO <sub>2</sub> /ZrO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub> /TiO <sub>2</sub> /ZrO <sub>2</sub>
Length	250 mm	250 mm	250 mm
Nominal MW Cut-Off	20 nm <sup>1,2</sup>	5 kD <sup>1</sup>	1 kD <sup>1</sup>

<sup>1</sup> as indicated by the manufacturer; <sup>2</sup> average pore size



**Fig.1:** Module and ceramic membranes (SEM-micrograph) used.

To determine adsorption capacity, defined volumes of the membranes were challenged in a static experiment with endotoxin-containing solutions at different concentrations ranging from 30 to 1000 EU/mL (i.e., endotoxin unit/mL). In all experiments endotoxins from *E. coli* (Serotype 055:B5) were used.

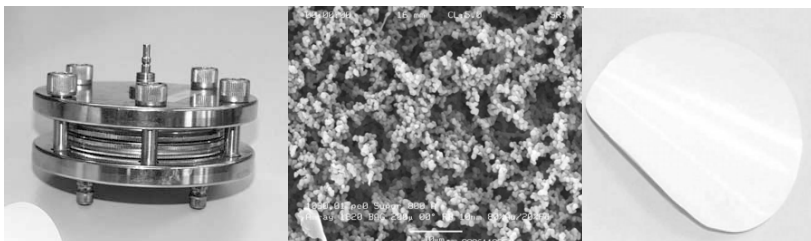
To investigate the recovery capacity during long-term runs, the membranes were challenged with endotoxin-containing solutions at concentrations ranging from 0 to 1000 EU/mL (i.e., endotoxin unit/mL) over a period of at least 24 h. In all experiments endotoxins from *E. coli* were used (*E. coli* Serotype 055:B5).

### Analytical method

Permeate samples were collected at intervals and assayed for endotoxins. Endotoxin concentration was measured with the kinetic turbidimetric Limulus Amoebocyte Lysate (LAL) Test. This method ensures sensitive detection of bacterial endotoxins, down to 0.125 EU/mL. In this investigation, reference shall be made to the upper limit of 0.25 EU/mL set for endotoxin concentration in dialysis water by the European Pharmacopoeia.

**Table 2:** Properties of the adsorber membranes used (as indicated by the manufacturer)

Adsorber Membranes		
Identity Functional group	Membrane #1 R-CH <sub>2</sub> -N <sup>+</sup> -(CH <sub>3</sub> ) <sub>3</sub>	Membrane #2 R-CH <sub>2</sub> -N <sup>+</sup> -(CH <sub>3</sub> ) <sub>3</sub>
Membrane height (mm)	0.08	0.06
Membrane volume with a diameter of the flat membrane of 9 cm (ml)	0.515	0.39
Average pore size (µm)	0.2	0.8
Average binding capacity	-	60 mg/ml (membrane volume) - BSA



**Fig.2:** Module and adsorber membranes (SEM-micrograph) used in this investigation.

### 3. Results and conclusion

In static experiments LPS adsorption was significant on polymeric adsorbive membranes, but minimal on ceramic membranes (Table 3).

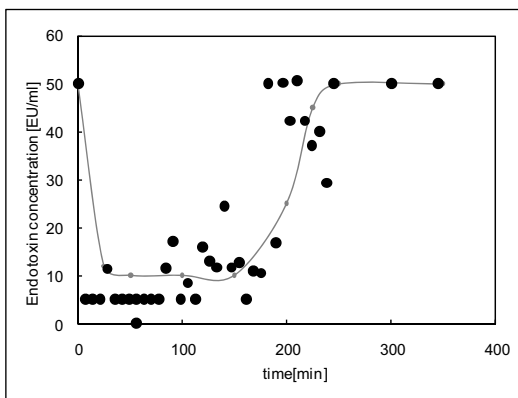
**Table 3:** Adsorbed endotoxin in units per ml membrane volume (EU/ml) after 24 h.

Endotoxin EU/ml	Adsorber membranes		Ceramic membranes		
	Membrane #1	Membrane #2	Membrane #1	Membrane #2	Membrane #3
	5600	2600	15	8	11

In dead-end filtration, significant LPS amounts permeated across ceramic membranes in the short term but were completely rejected in the long term (Table 4). In cross-flow filtration, none of the permeate produced with ceramic membranes meets the European standard, as shown earlier [4].

**Table 4:** Endotoxin concentrations in the permeate (EU/ml) after 1 h filtration across the membranes. NA: not available.

Endotoxin challenge [EU/ml]	Ceramic membranes			Adsorber membranes	
	Membrane #1	Membrane #2	Membrane #3	Membrane #1	Membrane #2
50	NA	NA	NA	1,5	0,75
100	<0,05	<0,25	<0,25	5	NA
1000	<0,05	<0,5	NA	NA	NA



**Fig.3:** Breakthrough of one layer of adsorber membrane # 1 (membrane diameter= 9 cm, membrane volume=0.515 ml) challenged with low LPS concentration in dead-end mode (50 EU/ml LPS).

Adsorber polymeric membranes are not able to keep the limit of 0,25 EU/ml even when challenged with low LPS concentrations, which may possibly be caused by binding site saturation (Figure 3).

The results show that different factors contribute to the endotoxin retention capacity of a membrane. Adsorption to charged moieties and hydrophobic interactions with the hydrophobic membrane backbone seem to dominate in ion-exchange polymeric membranes. However, pore size exclusion appears to play an important role in endotoxin retention by more chemically inert membranes.

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