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Membrane filtration enhanced by ultrasound for reducing endotoxin in dialysis water

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ABSTRACT

Lipopolysaccharide (LPS) endotoxin, a bacterial by-product, and potential dialysis fluid contaminant is a major concern in dialysis water treatment for the detrimental health effects it represents. This study aims to determine the influence of the ultrasonic-assisted filtration process on endotoxin reduction in dialysis water. In particular, ultrafiltration (UF) and nanofiltration (NF) membranes have been recognized for their capability to reduce endotoxin from synthetic dialysis water. An ultrasonic instrument, with a power of 350 W and a frequency of 40 kHz, was used in this study to enhance the capability of these membranes. A lab-scale unit was built to implement the experiments and synthetic water (feed solution) was prepared with a known level of endotoxin (0.48 EU/mL). The test for Limulus amebocyte lysate was used to assess concentrations of endotoxin in treated water. The experimental results showed significant changes in the ultrasonic (US) treatment of endotoxins when compared with both (US alone) and (US and UF), (US and NF), and (US, UF, NF). This kind of treatment reduced the concentration of endotoxin to 0.06 ± 0.06 EU/mL by enhancing membrane efficacy through ultrasonic treatment. The results of the study indicated that this could be an innovation in ultrasonic fields, with a wide range of prospects for making use in dialysis fluid preparation.

Keywords: Ultrasonic applications; Dialysis fluid; Bacterial endotoxin; Limulus amebocyte lysate (LAL) assay

1. Introduction

Dialysis water quality is one of the most important factors in ensuring a safe and effective delivery of hemodialysis (HD) [1]. For more than a decade, it has been suspected that there may be hazardous contaminants in the water and concentrates used to prepare dialysis fluid [2]. HD is the most common form of treatment for chronic renal failure, and it is usually done three times a week for 3–4 h, depending on the patient's clinical status [3]. Water is used in the manufacturing of dialysate as well as the reuse of dialyzers in this therapy, exposing patients to about 90–192 L of water per session through the semipermeable membrane during hemodialysis sessions [4]. As a result, the quality of the water utilized in this process is crucial to prevent putting patients' health at risk.

Endotoxins, which are lipopolysaccharides (LPS) and pyrogens, are an integral component of the outer membrane of gram-negative bacteria and thus ubiquitous in the environment. Endotoxins play an important role in

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the inflammatory and immune responses induced by gram-negative bacteria [5]. Therefore, the dialysis water disinfection procedure may cause bacterial cell wall breakdown, resulting in an immediate release of endotoxin and a significant increase in endotoxin activity in the water [6]. Although bacteria are frequently removed with a 0.2 μm sterilizing grade filter, LPS is difficult to remove or inactivate due to its extreme heat and pH stability [7].

The dialysis water treatment system employs several devices and processes for adequate water treatment to eliminate different substances with different modalities applied in series [6]. Ultrafiltration (UF) is one membrane filtration process that serves as a barrier to separate harmful bacteria, viruses, and other contaminants from clean water [8]. But the membrane fouling is the primary issue in membrane filtration operations, resulting in a decrease in permeate flux over time and a limitation in separation efficiency. The formation of a fouling layer on the porous membrane surface has a significant impact on the performance of the membrane in crossflow filtration. When a contaminant coats the membrane surface, it reduces the rate of water transport across the membrane [9].

This prospective study tested whether improved dialysis water purity by an additional ultrasonic (US) disinfection is a popular, eco-friendly disinfection method that produces no disinfection by-products. It is a chemical-free technique that causes cavitation in the solution, which causes cell disruption. Bubbles form and break, generating turbulence and pressure changes that can rupture bacteria [10]. However, using US disinfection treatment alone not only makes complete disinfection difficult but also requires a significant amount of energy for large-scale disinfection treatment [11]. As a result, the US disinfection in conjunction with other technologies in the field of reducing endotoxin in dialysis water disinfection must be researched.

The guidelines set by the American National Standards Institute, the Association for the Advancement of Medical Instrumentation, and the International Organization for Standardization (ANSI/AAMI/ISO) have established maximum limits for chemical contaminants, bacterial colony-forming units, and endotoxin levels. Some contaminants, however, such as fungus and algae, are still not present in dialysis fluids [12].

In this study, a combination of two or three processes; ultrasonic disinfection, and ultra/nanofiltration membranes will be examined to investigate their efficiencies for endotoxin removal and the role of ultrasonic in enhancing the efficacy of membranes.

2. Materials and methods

2.1. Work strategy

Water samples were prepared synthetically in the Iraqi Ministry of Science and Technology in Baghdad. Limulus amebocyte lysate (LAL) reagents were used to prepare standard endotoxin control (CSE) *E. coli* 500 ng/vial at a concentration of 1,000 EU/mL to be used with the gel clot endpoint method by Limulus amebocyte lysate (LAL) reagents, which are engineered to detect the presence of bacterial endotoxins [13]. After that, at room temperature, this solution was mixed with double-pass RO water with an endotoxin concentration of <0.06 ± 0.06 and an electric conductivity of 0.5 μS/cm to produce synthetic water (feed solution) with an endotoxin content of 0.488 ± 0.06 EU/mL that will be utilized in all tests. This endotoxin concentration is higher than the ANSI/AAMI/ISO dialysis water guideline, and it falls within the range of endotoxin concentration values previously discovered in dialysis water studied from many dialysis centers in Iraq [14]. All glassware was heated at 350°C–400°C for at least 30 min to remove all pyrogen [15]. Some items, such as dilution tubes, micropipetter tips, and aluminum caps were purchased without pyrogens. The experiments were divided into 3 groups: group A, ultrasonic experiments only; group B, ultrafiltration after ultrasonic; and group C, ultrafiltration and nanofiltration after ultrasonic.

2.2. Ultrasonic experiments design

The ultrasonic cleansing equipment was prepared after being washed before use by changing the distilled water in the reservoir without the addition of a disinfectant solution. The container of the feed solution (dialysis water) was placed in a stainless steel tank, equipped with a digital ultrasonic device (Model LUC405 from Daihan Labtech ultrasonic Instrument Co., Ltd., Korea) at a constant power of 350 W and frequencies of 20, 30, and 40 kHz. Several preliminary experiments were conducted to obtain the optimal frequency of 40 kHz [8] and the amplitude of the instrument considering operating parameters established in previous studies [16]. A series of experiments involved the use of high-frequency sonication of endotoxin and monitoring the effect on its reduction. The gel clot assay was used to assess endotoxin in samples obtained from the ultrasonic reactor at various points during the US treatment (time = 0, 10, 20, 30, 40, 50, and 60 min). A series of experiments were carried out to maintain the experimental condition at a power density of 350 W/L (expressed as ultrasonic power/volume for the ultrasound bath).

2.3. US with UF and NF experiments design

A small lab-scale skid (Fig. 1) from Sterlitech Corporation-USA with a dead-end used two types of commercial membranes for ultra and nanofiltration. A commercial ultrafilter (UF) membrane polymer with a molecular weight cut-off (MWCO) of 30 kDa was used to compare the performance of polyamide thin-film nanofiller (NF) membranes with MWCOs ranging from 150 to 300 Da. The experiments were started by purifying the skid with pure multi-pass RO water that has an endotoxin concentration of ≤0.06 EU/mL with almost zero pressure to remove any suspended in the tank [17]. Then the emptied tank was filled with 300 mL of feed solutions, followed by continuing filtering. A digital controller was used to set the transmembrane pressure.

2.4. Statistical analysis

An analysis of variance (ANOVA) test was used to determine the significant differences among the three

Fig. 1. Photograph of Sterlitech bench scale and a Power-Sonic 405 (5 L) ultrasonic cleaner for water treatment.

methods at a *P*-value of 0.05. The Data Analysis package in Microsoft Excel 2010 was used to perform the single-factor ANOVA analysis.

3. Results

3.1. US treatment

Synthetic feed water samples were disinfected using US treatment to eliminate endotoxin at different contact times (10, 20, 30, 40, 50, and 60 min) with temperatures ranging from 23°C ± 0.1°C to 36.9°C ± 0.70°C. The results are summarized in Table 1.

Except for a relatively long time of 60 min, the data show that short-term exposure to US waves does not affect endotoxin concentration. At this exposure time, the reduction was small (0.339 ± 0.09 EU/mL), and this is still higher than the standard guideline value of 0.25 EU/mL.

3.2. Hybrid treatment (US and UF)

In this case, the results of treating feed water with ultrasonic and ultrafiltration (US and UF) as a hybrid treatment are compared to those obtained with UF alone at 4 bar pressure and 25°C, and to US treatment alone at the same contact times. The comparison is shown in Table 1.

When compared to US treatment alone, this treatment is more effective at reducing endotoxin concentrations. The endotoxin content was 0.48 ± 0.06 EU/mL at the start of the experiments. US treatment alone did not reduce the endotoxin concentration till after 60 min of the treatment time (0.339 EU/mL). Similarly, UF treatment alone reached the same concentration (0.339 EU/mL). The optimal contact time for US treatment with UF to reduce endotoxin concentration to 0.24 ± 0.06 EU/mL was achieved at 40 min. This concentration complies with the international guideline for endotoxin value (0.25 EU/mL). The long US treatment increases the water temperature, and this could jeopardize the efficiency of the membrane to filter the endotoxins and could be the reason behind getting flat concentration values as time goes higher. Statistically, there was a significant difference between the treatment with the (US and UF) and the treatment with US alone at ($P < 0.05$), where *P*-value = 0.30%.

3.3. Hybrid treatment (US and NF)

Another double treatment was employed by using both ultrasonic and nanofiltration US and NF as a hybrid treatment to reduce endotoxin concentration. These results were compared to those obtained when using NF only at 8 bar pressure and 25°C. Table 2 shows the comparison.

Using the NF treatment alone was capable of reducing the endotoxin concentration to below the international guideline value (0.24 EU/mL). However, the highest reduction of endotoxin concentration was achieved when using this combination, and the endotoxin concentration was reduced to 0.12 ± 0.06 EU/mL at a contact time of 50 min. Statistical analysis showed a significant difference between the treatment with the (US and NF) and both the treatments with the US alone or NF alone at ($P < 0.05$).

3.4. Triple treatment (US, UF, NF)

Table 3 shows the water quality after a triple treatment process for feed water solution to reduce endotoxin concentration, using US, UF, and NF at a contact time ranging from 0 to 60 min and temperatures ranging from 23°C ± 0.45°C to 36.4°C ± 0.50°C at 8 bar pressure.

This treatment was more efficient compared to the other treatments mentioned above. The best result for water was obtained at a contact time of 40 min, where the main endotoxin concentration was decreased to 0.06 ± 0.06 EU/mL, which is well below the permissible international standard

Table 1
Results for US and UF treatment for feed water to reduce endotoxin from the initial concentration of (0.48 EU/mL)

Test no.	Control of endotoxin concentration (EU/mL)	Contact time (min) for US	Temperature, °C	Endotoxin concentration (EU/mL)		
				US	UF	US and UF
1		0	23 ± 0.1	0.48 ± 0.06		0.339 ± 0.09
2		10	24.2 ± 1.1	0.48 ± 0.06		0.339 ± 0.09
3		20	26.5 ± 2.1	0.48 ± 0.06		0.339 ± 0.09
4	0.48 ± 0.06	30	29.5 ± 1.3	0.48 ± 0.06	0.339 ± 0.09	0.339 ± 0.09
5		40	32.2 ± 0.9	0.48 ± 0.06		0.24 ± 0.06
6		50	34.2 ± 0.8	0.48 ± 0.06		0.24 ± 0.06
7		60	36.9 ± 0.70	0.339 ± 0.09		0.24 ± 0.06

Table 2
Results for US and NF treatment for feed water to reduce endotoxin from the initial concentration of (0.48 EU/mL)

Test no.	Control of endotoxin concentration (EU/mL)	Contact time (min) for US	Temperature, °C	Endotoxin concentration (EU/mL)	
				NF	US and NF
1		0	23.8 ± 0.1	0.24 ± 0.06	0.24 ± 0.06
2		10	25.3 ± 0.1		0.24 ± 0.06
3		20	27.1 ± 0.3		0.24 ± 0.06
4	0.48 ± 0.06	30	29.5 ± 0.14		0.24 ± 0.06
5		40	32.3 ± 0.5		0.169 ± 0.09
6		50	34.6 ± 0.1		0.12 ± 0.06
7		60	36.2 ± 0.2		0.12 ± 0.06

Table 3
Results for triple treatment for feed water to reduce endotoxin from the initial concentration of (0.48 EU/mL)

Test no.	Control of endotoxin concentration (EU/mL)	Contact time (min) for US	Temperature, °C	Endotoxin concentration (EU/mL)	
				UF and NF	US, UF, and NF
1		0	23 ± 0.45		0.169 ± 0.09
2		10	24.5 ± 1.4		0.169 ± 0.09
3		20	25.8 ± 2.3		0.169 ± 0.09
4	0.48 ± 0.06	30	28.35 ± 0.9	0.169 ± 0.09	0.12 ± 0.06
5		40	32.3 ± 0.56		0.06 ± 0.06
6		50	33.9 ± 0.6		0.06 ± 0.06
7		60	36.4 ± 0.50		0.06 ± 0.06

value of 0.25 EU/mL [12]. The statistical analysis showed significant differences among treatments at ($P < 0.05$). Table 4 shows a sample output of the ANOVA analysis for this case, in which it is shown that the obtained P-value is very small (0.00003).

4. Discussion

Endotoxin reduction was investigated after using high-frequency (40 kHz) ultrasonic (US) treatment. US treatment alone did not comply with the international standard guideline value of endotoxin concentration (0.25 EU/mL), with an ultimate concentration after 60 min of treatment reaching 0.339 EU/mL. Accordingly, it is not recommended to use this treatment for dialysis water. For this reason, it is important to use a new method that can give us the best results with a shorter contact time for ultrasonic treatment.

A previous study [18] showed that after UF, endotoxin concentrations in dialysis water samples decreased from an average value of 0.44 to 0.013 EU/mL. Another study used a standard water treatment to produce dialysis water with an endotoxin content of 0.125 EU/mL and then used ultrafiltration to achieve an endotoxin concentration of zero EU/mL [19]. Repeated membrane sterilization, on the other hand, degrades the membrane separation properties and limits their use by combining filtration and adsorption [2].

In this study, the results of using US treatment together with the ultrafiltration membrane showed that UF treatment alone did not reduce the endotoxin concentration below the standard guideline value while enhancing the treatment by ultrasonic treatment for 40 min can reduce endotoxin concentration of the synthetic dialysis water to 0.24 EU/mL, as shown in Table 1.

The study showed in Table 2 that when dialysis water containing endotoxins is passed directly through the NF membrane alone, this will increase the possibility of a fouling phenomenon due to the formation of a trapped substance on the surface of the membrane or within the membrane pores, accumulation of these foulants will lead to an increase in resistance to the permeate flux. A substance accumulates when the permeation force – the force that moves potential impurities towards the membrane – is greater than the forces acting in the opposite direction (i.e., away from the membrane) [20]. In addition, the accumulation of pollutants inside the pores of the membrane, and due to the high operating pressure, can cause a change (increase) in the size of the pores and thus reduce the efficiency of the membrane in removing pollutants [21].

Furthermore, in a previous study by Czermak et al. [18], it was discovered that nanomembranes are unable to reduce the limit of 0.25 EU/mL even when challenged with low LPS concentrations, which could be due to binding site saturation and separation processes through a sieving mechanism based on the MWCO [19].

The results in Table 3 showed that combining endotoxin removal processes revealed that the ultrasonic with UF and NF sets could lead to substantial removal of endotoxin, reaching 0.06 EU/mL. However, the additional ultrasonic instruments for conventional treatment, on the other hand, will raise the investment cost of the dialysis treatment unit. Nonetheless, ultrasonic disinfection is safer, extends the

Table 4
A single-factor ANOVA analysis was applied for the comparison of the three techniques (ultrasonic, ultrafilter, and nanofilter)

Summary						
Groups	Count	Sum	Average	Variance		
US and UF	7	2.076	0.296571	0.0028		
US and NF	7	1.375	0.196429	0.003191		
US, UF, and NF	7	0.807	0.115286	0.002975		
ANOVA						
Source of variation	SS	df	MS	*F*	*P*-value	*F*-crit
Between groups	0.115447	2	0.057723	19.31382	3.31E-05	3.554557
Within groups	0.053797	18	0.002989			
Total	0.169244	20				

lifespan of the membrane, reduces health risks, and lowers the operating costs of the treatment plant. As a result, it is important to adopt a new design to modify the currently operational dialysis water treatment units in Baghdad to produce pure water for HD applications that meet international dialysis water quality standards and save patients' lives.

Finally, this study concluded that with the use of triple treatment by combining (US/UF/ NF) a large part of the pollutants were removed in the UF stage (which works according to the sieve's mechanism) thus reducing the load on the NF membranes (which operates according to the osmosis mechanism) in removing the dissolved pollutants that were not rejected in the UF stage and thus improving the performance of the NF membranes in removing the bulk of the endotoxins remaining.

5. Conclusion

Endotoxins are reduced by ultra/nanofiltration membranes but are not completely eliminated. The ultrasonic treatment of water in conjunction with the membranes was effective in further reducing endotoxin concentrations in synthetic dialysis water. Although the addition of ultrasonic instruments increases the investment cost, it can help to reduce the running cost by decreasing the membrane maintenance and extending the lifespan of the membrane through the dispersion of contaminants.

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Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this manuscript.

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