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The role of controlling zeta potential for endotoxin removal in dialysis water preparation

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ABSTRACT

Hemodialysis (HD) centers in Baghdad show high endotoxin concentrations. This reflects the removal of endotoxin was not under consideration in the dialysis units. The objective of this research is to evaluate several treatment processes to eliminate free endotoxin released from the ruptured cell wall of gram-negative bacteria in dialysis water. The studied treatment options include ultraviolet (UV), ultrafiltration membrane (UF), and zeta potential control was employed to improve the efficacy of the UF. Hybrid treatment was also considered by joining two or more of the above treatments. 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 observed experimental results showed significant changes in the zeta potential of endotoxins when compared with both (UV alone) and (UV and UF). This kind of treatment reduced the concentration of endotoxin to 0.24 EU/mL by enhancing UF efficacy through zeta potential control and UV treatment. This study serves as a basis for applying physical treatment methods to the currently used water treatment techniques to produce dialysis water in compliance with the international dialysis fluid quality standards.

Keywords: Bacterial endotoxin; Dialysis water; Water treatment; Ultrafiltration membrane; Ultraviolet; Zeta potential

1. Introduction

In a dialysis center, hemodialysis is usually done twice or three times a week for about 4 h per patient. Accordingly, patients undergoing hemodialysis are usually exposed to very large amounts of water more than 90–192 L per session [1]. Therefore, the quality of water used for dialysis is very important in preventing chemical and bacteriological contaminants of diffusion from dialysis fluids into the bloodstream of patients [2]. Furthermore, such contamination of dialysis water can be a possible cause of high mortality for dialysis patients [3].

Municipal water is the main source of water used in dialysis centers, which passes through several pretreatment levels in order to eliminate contaminants and to produce hemodialysis water [4]. Hemodialysis water requires additional treatment to reduce the exposure of patients to potential contaminants that are present in the feed water or those created during the water treatment processes [5].

Endotoxin (pyrogenic fractions) is a lipopolysaccharide (LPS) mostly coming from gram-negative bacteria cell wall component that is released during cell death or cell growth [6], easily passes through a dialysis membrane that induces blood cell activation [7].

The guideline set by the American National Standards Institute ANSI/AAMI endotoxin concentration <0.25 EU/mL, while the standard limits of ultrapure dialysate are <0.03 EU/mL for dialysis fluid [8]. This value will be used

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to compare the results of this research to evaluate the effect of controlling zeta potential on the quality of the produced dialysis water.

The process of dialysis water disinfection may break down bacterial cells wall resulting in the immediate release of endotoxin and significantly increase the level of endotoxin activity in the water. As a result, the performance of disinfection could lead to the generation of a potential risk of free endotoxin, which could be considered a by-product of disinfection [9]. Although intact bacteria can be captured using a 0.2 μm filter, the LPS can be more challenging [10].

Zeta potential (surface charge) has been used in water treatment processes for many years to help determine coagulant dosages that depend on the charge of the suspended particles, pH, and conductivity [11]. Zeta potential is a measure of the electrical potential between particles, showing the repulsive interaction between particles; a zero zeta potential means that the particles in the water are unstable, that is, the conditions for aggregation are maximized [12].

In the present study, a combination of two disinfection processes; ultraviolet (UV), and ultrafiltration membrane (UF) will be examined to investigate their efficiencies for endotoxin removal and the role of controlling zeta potential on their performance.

2. Materials and methods

2.1. Endotoxin test

For the evaluation of the bacterial endotoxins test, a Limulus amebocyte lysate (LAL) was used. The gel-clot method is utilized to recognize or quantify endotoxins based on the clotting of the lysate reagent in the presence of endotoxin, after an incubated at 37°C ± 1°C for 60 ± 2 min without vibration [13].

2.2. Synthetic water preparation

Wako Chemicals Inc., (USA) reagents have been used to prepare standard control of endotoxin *Escherichia coli* (500 ng/vial) at a concentration (1,000 EU/mL) used by LAL with the gel clot endpoint method [13]. This solution was then mixed with ultrapure multi-pass reverse osmosis water with an electrical conductivity of 0.5 μS/cm to obtain the synthetic water (feed solution). This feed solution was used in all experiments with a reference endotoxin concentration of (≥0.48 EU/mL), which is higher than the ANSI/AAMI guideline value for dialysis water. All these solutions were prepared at the laboratories of the Iraqi Ministry of Science and Technology, Baghdad.

All the preparation glassware was rendered endotoxin-free by heating in an oven at 350°C–400°C for 30 min [14,15]. Depyrogenated dilution glass tubes, depyrogenated reaction glass tubes, and depyrogenated tips for pipettes were purchased from Wako Chemicals Inc., USA.

2.3. UV and UF experiments

UV and UF experiments were conducted using low-pressure UV lamps (Electronic Ballast, 6 W). The irradiance of this light from the UV lamp was (38.21 mW/cm_2) at UV dose (4.585, 9.17, and 18.34 mJ/cm_2). A membrane cell module (SEPA CF from Sterlitech Corporation, Kent, WA) with a polyethersulfone membrane with a size of 190mm × 140 mm and molecular mass cut-off of 20 kDa was used in all experiments. The samples were pumped using (Hydracell pump) through the membrane module at a pressure of 2 bar. UF experiments were used alone in a previous study by Humudat et al. [16]. In this study, a UV treatment setup upstream of the UF membrane treatment was employed. Fig. 1 shows a photograph of the lab-scale skid used in this study.

2.4. Zeta potential experiment

All zeta potential control experiments were implemented using zeta potential control rod from (Zeta Rod model ZRS-20 from Zeta Corporation). A zeta potential measurement was carried out using a zeta plus instrument (zeta potential analyzer) manufactured by Brookhaven

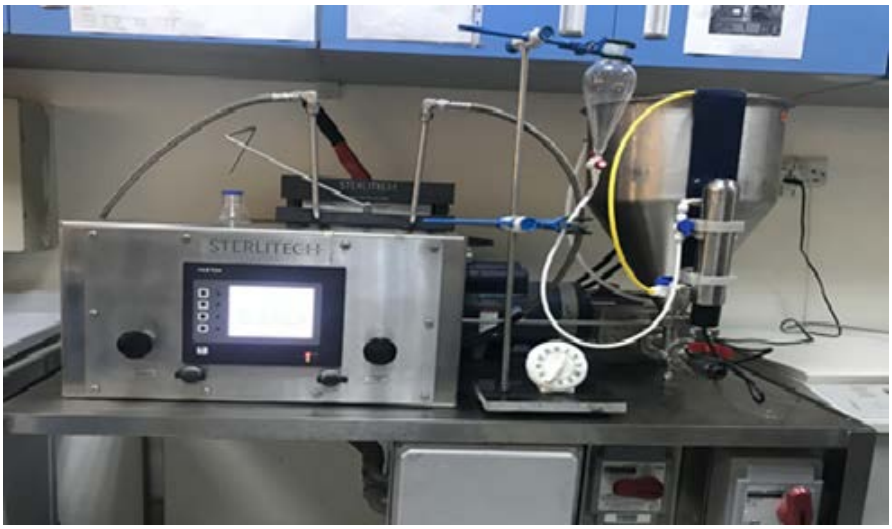

Fig. 1. Lab-scale UV disinfection with UF membrane testing skid.

Co., USA. Zeta potential measurements were carried out in disposable capillary cells. Samples were analyzed immediately after withdrawal from the zeta rod reactor after reaction times ranging from zero to 60 min. This method requires a very small sample (about 1 mL of water) and is relatively automated in the program so that a standard operating procedure can be incorporated. Fig. 2 shows a picture of both the zeta plus and zeta rod instruments used in this study.

2.5. Statistical analysis

Data were analyzed using the Microsoft Excel program with a *t*-test hypothesis of equal variance, double-tailed for the comparison of each two sets of obtained results. The marginal significant value (*P*-value) for this method was set to ($P < 0.05$).

3. Results

3.1. UV treatment

Synthetic feed water samples were disinfected using UV treatment to remove endotoxin at a contact time ranging from 2 to 8 min. The results are shown in Table 1.

The results showed no effect of UV exposure on endotoxin concentration, only after a relatively long time of 8 min. In this case, the reduction was relatively small (0.34 ± 0.09 EU/mL), which is still higher than the guideline standard value of (0.25 EU/mL).

3.2. UV and UF treatment

In this, case a combination of UV and UF as a hybrid treatment to reduce the concentration of endotoxin was conducted. Results are shown in Table 1 for comparison with those obtained when using UV alone at the same contact times. The result showed a reduction of endotoxin concentration when using this combination and the endotoxin concentration was reduced to (0.34 ± 0.09 EU/mL) at a contact time of 4 min. Increasing contact time for more than 4 min did not improve the efficiency of the UF to reduce the endotoxin concentration. Statistical analysis showed no significant differences in both treatment methods at ($P < 0.05$).

3.3. Role of zeta potential control

In order to evaluate the effect of controlling zeta potential, the zeta rod instrument was used after the UV disinfection and upstream of the UF membrane. The exposure period to the zeta rod was varied from zero to 60 min. Table 2 shows the results of introducing zeta potential control on the endotoxin removal efficiency when using the UV and UF treatment as described previously.

The result showed an increase of zeta potential value of water samples from (–31.76 ± 0.43 mV) when no zeta potential control was applied to (–20.97 ± 2.85 mV) when 60 min exposure in zeta potential control reactor. This increase reduces the electric potential among particles, and thus maximizing the aggregation ability of contaminants in water samples, and thus giving endotoxins better opportunity swept with them.

The results showed that the highest UF efficiency to decrease endotoxin concentration up to (0.24 ± 0.06 EU/mL) was achieved at high zeta potential value (≥24 mV). This was achieved when the exposure time was 30 min or higher. The endotoxin concentration in this case complied with the acceptable endotoxin concentration guideline values [8].

Statistical analysis showed significant differences among triple treatment (i.e., UV, UF with zeta potential control) with both (UV alone) and treatment with (UV and UF) at ($P < 0.05$), where *P*-values = 2.56% and 4.37%, respectively. Overall, these results emphasize the role of increasing the surface charges has a sensible effect in reducing the endotoxin concentration in dialysis water, depending on the exposure contact time for zeta potential and UV treatment.

4. Discussion

The effects of some water treatment options on endotoxin removal from dialysis water have been studied. This result of using UV alone clearly demonstrates that the installation of a UV lamp does not significantly remove the endotoxin from the prepared synthetic water. This result is in agreement with the useless effect of UV in the dialysis of ultra-pure RO water plants reported by Xue et al. [9], who stated that the UV alone cannot significantly remove the endotoxin activity because the LPS contains a lot of saccharide rings and lipid backbone, but no aromatic ring. Thus, there is no potential chemical absorption group of

Fig. 2. Zeta plus (a) and zeta rod (b) instruments used to measure and control zeta potential.

Table 1
Results for UV 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)	Endotoxin concentration (EU/mL)	
			UV	UV and UF
1	0.48	0	0.48 ± 0.06	0.48 ± 0.06
2	0.48	2	0.48 ± 0.06	0.48 ± 0.06
3	0.48	4	0.48 ± 0.06	0.34 ± 0.09
4	0.48	8	0.34 ± 0.09	0.34 ± 0.09

UV-254 in the molecule group that renders the endotoxin undisturbed. In contrast, Ren et al. [17] have claimed that when UV lamps are used in the RO water, their favorable effect is to prevent bacteria and destruct endotoxin. Also, in a previous study by Anderson et al. [15] for endotoxin inactivation in drinking water, this found UV dose between 40 and 100 mJ/cm$_2$ capable of inactivating endotoxin levels from the initial concentration range as high as (1–50 EU/mL) found in untreated water to 0.55 EU/mL.

Nevertheless, it is widely claimed that UV irradiation destructs endotoxin and thus increases water endotoxin levels. While no direct evidence supports this hypothesis, the removal of endotoxin by UF after UV irradiation is often recommended [18].

The combination of UV and UF treatment is more efficient in reducing endotoxin concentration compared to UV treatment alone. Anderson et al. [15] found that endotoxin inactivation was proportional to the UV dose under their tested conditions, while the UF method depends mainly on the pore size of the membrane to perform particulates separation based on their sizes [19].

In a previous study by Sam [20], it was found that a reverse osmosis filter is most widely used to eliminate more than 95% of the remaining ions and some bacteria. In this case, however, bacteria are not totally removed, and this requires the use of ultrafilter or UV radiation to destroy them. Dialysis water refers to even more strict limits on bacteria counts and bacterial toxin levels which are accomplished by specialized filters, not employed routinely at most dialysis centers in Iraq.

The results in Table 2 showed that endotoxin concentration reduction was enhanced by zeta potential control. Most organic molecules have a negative charge and are attracted to the positively charged media or membrane. Charged media can be more effective in removing endotoxin from a fluid [10]. UV exposure ruptures the cell wall of gram-negative bacteria and causing the release of a fragment of the cell wall in a form of free endotoxins into water. Bacteria are removed by submicron filters, but endotoxins are not removed [5]. Therefore, zeta potential may aid in their ability to remove endotoxin through the aggregation of those fragments (endotoxin) that can be captured by UF. This suggests that controlling zeta potential provides enhanced retention of fine particles smaller than the membrane's rating.

This previously unreported finding is significant because the use of a physical treatment like zeta potential control is safe and economical since it adds no chemicals and the instruments consume very low energy in the water treatment processes.

5. Conclusion

All types of treatment processes applied in this study have improved the purity of dialysis water and reduced endotoxin levels. Zeta potential values are key parameters in reducing endotoxin concentration in the dialysis water treatment processes. Accordingly, it is necessary to consider a new design to modify the currently working dialysis water treatment units in Baghdad to produce safe water for dialysis applications that comply with the international standards of dialysis water quality and save patients' lives.

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Table 2
Effect of zeta potential value (mV) on the endotoxin concentration reduction (EU/mL)

Test no.	Control of endotoxin concentration (EU/mL)	UV cont. time (min)	Endotoxin concentration (EU/mL) with exposure time for zeta potential control of			
			0 min	15 min	30 min	60 min
1	0.48	0	0.48 ± 0.06	0.48 ± 0.06	0.48 ± 0.06	0.48 ± 0.06
2	0.48	2	0.48 ± 0.06	0.48 ± 0.06	0.48 ± 0.06	0.34 ± 0.09
3	0.48	4	0.34 ± 0.09	0.34 ± 0.09	0.34 ± 0.09	0.24 ± 0.06
4	0.48	8	0.34 ± 0.09	0.34 ± 0.09	0.24 ± 0.06	0.24 ± 0.06
Zeta potential (mV)			–31.76 ± 0.43	–28.39 ± 1.47	–23.99 ± 2.11	–20.97 ± 2.85

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