Enhancement of Toxic Substances Clearance from Blood Equvalent Solution and Human Whole Blood through High Flux Dialyzer by 1 MHz Ultrasound

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ABSTRACT

Background: Hemodialysis is a process of removing waste and excess fluid from blood when kidneys cannot function efficiently. It often involves diverting blood to the filter of the dialysis machin to be cleared of toxic substances. Fouling of pores in dialysis membrane caused by adhesion of plasma protein and other toxins will reduce the efficacy of the filtre.

Objective: In This study, the influence of pulsed ultrasound waves on diffusion and the prevention of fouling in the filter membrane were investigated.

Material and Methods: Pulsed ultrasound waves with frequency of 1 MHz at an intensity of 1 W/cm^2 was applied to the high flux (PES 130) filter. Blood and blood equivalent solutions were passed through the filter in separate experimental setups. The amount of Creatinine, Urea and Inulin cleared from both blood equvalent solution and human whole blood passed through High Flux (PES 130) filter were measured in the presence and absence of ultrasound irradiation. Samples were taken from the outlet of the dialyzer every five minutes and the clearance of each constituent was calculated.

Results: Statistical analysis of the blood equvalent solution and whole blood indicated the clearance of Urea and Inulin in the presence of ultrasound increased ($p<0.05$), while no significant effects were observed for Creatinine.

Conclusion: It may be concluded that ultrasound, as a mechanical force, can increase the rate of clearance of some toxins (such as middle and large molecules) in the hemodialysis process.

Keywords

Ultrasound, High Flux Dialyzer, Creatinine, Urea, Inulin

Introduction

Healthy kidneys clean blood and remove extra fluid in the form of urine. Hemodialysis (HD) is a medical treatment in which dialysis is used to remove poisons and toxins from a patient's blood. Blood is obtained from the patient using needles and plastic tubing. The blood is then pumped through a semipermeable dialysis membrane. Poisons and toxins whuch are usually removed by kidneys pass through the membrane by diffusion into a liquid called dialysate. The di-

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alysate is discarded along with the toxins, but the purified blood is returned to the patient's body [1].

The most important part of a HD machine is the dialyzer filter or artificial kidney. It is a cylinder that contains very thin fibres that act as a dialysis membrane. Blood runs along the middle of the fibres and the dialysis fluid is pumped on the outside of the fibres. There is no contact between the blood and the dialysate. In the dialyzer, toxins and excess water (which are the equivalent of the urine produced by the healthy kidneys) pass from the blood into the dialysate. In the dialyzer, the blood flows along one side of a semipermeable membrane made of cellulose or a similar product, with the dialysate flowing on the other side. Different molecules pass through dialyzer, at different rates, until a suitable concentration is achieved [2]. One reason for the decline in the dialysis membrane filtration is fouling of pores in the dialyzer during the filtration, leading to long hemodialysis duration [3].

Fouling has sometimes been seen to reduce the active area of the membrane which therefore leads to a reduction in permeate flux below the theoretical capacity of the membrane for the given driving force [4]. The reduction occurs due to the buildup of materials (adsorbed macromolecules, gel formation, pore blockage and deposited particles on or in the membrane surface) and concentration polarization; this leads to an accumulation of particles or solutes in a mass transfer boundary layer adjacent to the membrane surface [5].

In order to control concentration polarization and fouling to increase the permeate flux, techniques such as gas sparging, vibration, back- and cross-flushing have been utilized. In recent years, much attention has been paid to the use of auxiliary forces to increase the membrane efficiency of filtration. Considering many useful applications of ultrasound in various biomedical areas, one may select it as an external force to various dialysis membranes [6-9]. Many studies have reported the advantages of membrane cleaning by ultrasound with frequency of 28–500 kHz over ultrafiltration and microfiltration membranes [10-21]. Little research is available regarding hollow-fiber membrane. One of the few studies on these membranes which has been done with both online continuous and offline pulse ultrasonic irradiation indicated their effectiveness on the fouling mitigation [22].

Tatsumoto et al. conducted a rudimentary test to investigate the increase of the efficiency of dialysis by sonication. They reported a remarkable enhancement of the efficiency of dialysis (1.5 folds) in their experiments [23].

Various studies on the influence of ultrasound frequencies from 28 kHz up to 1 MHz on the fouling in membrane showed that lower frequencies have had better cleaning efficiencies than higher frequencies [11]. Since our test is performed on human whole blood, we use a pulsed ultrasound frequency of 1 MHz to reduce the formation of cavitation. According to many reports, increasing the frequency and also decreasing the intensity of ultrasound reduces the formation of cavitation in liquids and its activity in the field [11].

Objectives

The aim of this study is to investigate the effect of pulsed ultrasound irradiation on high flux dialyzer. Current study compares the clearance of Urea, Creatinine and Inulin in the presence and absence of ultrasound irradiation in blood equivalent liquid and human whole blood.

Material and Methods

All chemiacals used in this study were purchased from Merck company (Germany) and utilized without any extra purification. A pulsed ultrasound generator with three piezoelectric transducers operating at a frequency of 1 MHz and the nominal intensity of 1 W/cm^2 was designed in the department of Medical Physics at Iran University of Medical Sciences (IUMS). Each transducer has an area of

4 cm^2 and pulses at 0.3 second consecutively. The transducers were fixed on a Plexiglass frame. The transducer housing was then kept at 1 cm from the housing of dialyzer with water between them as matcing layer.

A high flux filter with polyethersulfone hollow fiber membrane (PES 130, 1.3 m2 surface area, 200±15 µm inner diameter and 30±5 µm wall thickness) was used which is commercially available on the market provided by Meditechsys company. Each filter consists of 8300±100 fibers bundeled together with 283±2 mm length. The molecular weigth cut-off (MWCO) for PES 130 dialyzer used in this study was more than 10 kDa. The experiments were carried out in two parts; First with blood equivalent solution and then with the human whole blood:

Blood Equivalent Solutions

The blood equivalent solution was prepared with the following constitutents: Urea with a concentration of 0.6 gL^{-1}, Creatinine with a concentration of 0.1 gL^{-1}, Dinatriumhydrogenphosphat- dihydrat ($Na_2HPO_4 \cdot 2\ H_2O$) with a molecular weight of 177.99 Da and concentration of 0.23 gL^{-1}, Natriumdihydrogen phosphat-dihydrat ($NaH_2PO_4 \cdot 2\ H_2O$) with a molecular weight of 156.02 Da and concentration of 0.05 gL^{-1}, Inulin with concentration of 0.2 gL^{-1}. The dialysate was prepared by the mixture of sodium chloride (Nacl) with a concentration of 9 gL^{-1} in distilled water.

Human Whole Blood

For the seconed part of this study, 36 bags of human whole blood with the same blood groups were received from Iranian Blood Transfusion Organization (IBTO). The concentrations of serum Urea (CH_4N_2O) with a molecular weight of 60.06 Da and Creatinine ($C_4H_7N_3$) with a molecular weight of 113.12 Da were measured. The received blood belonged to healthy individuals, thus in order to simulate with the blood of renal failure patients, the measured Urea and Creatinine levels increased to the mean value of levels in patients undergoing dialysis. Urea and Creatinine concentrations should reach 1 gL^{-1} [24] and 0.1 gL^{-1} [25], respectively. Also, Inulin ($C_6H_{10}O_5$) with a molecular weigth of 5200 Da as an important indicator in dialysis with concentration of 0.2 gL^{-1} was added to the blood. The dialysate was prepared by mixture of sodium chloride (Nacl) with a concentration of 9 gL^{-1} in distilled water.

Experimental Setup

Experimental setup is illustrated in Figure 1. The blood equivalent solution and human whole blood were discharged seperately in a plastic container placed inside water heater tank 1 and dialysate was poured in water heater tank 2. The temperature of both solutions were kept constant at 37°C, similar to human body temperature. Blood and dialysate flow rate in connector tubes (PVC) were 200 (ml/min) and 500(ml/min), respectively. The flow dynamics of blood and dialysate from two different tanks were accompolished by the use of two peristaltic pumps (Heidolph pump drive 5206, GERMANY). It was made sure that during the experiment the pressure in the flow circuit stayed constant. Samples of blood were taken from the outlet of the dialyzer every five minutes for 60 minutes. The experiments with PES 130 dialyzer with and without ultrasound were carried out. The experiments started with PES 130 dialyzer without ultrasound. The samples were taken according to the protocol mentioned above. The same procedure was carried out for the second part of the experiments in the presence of ultrasound. Blood samples were taken from the outlet of the dialyzer every five minutes for 60 minutes period of dialysing process.

Clearance Calculation

Concentration of all substances in blood equivalent liquid and whole blood was measured by a spectrophotometer (GENESYS 10 UV, Thermo, USA). Using the following

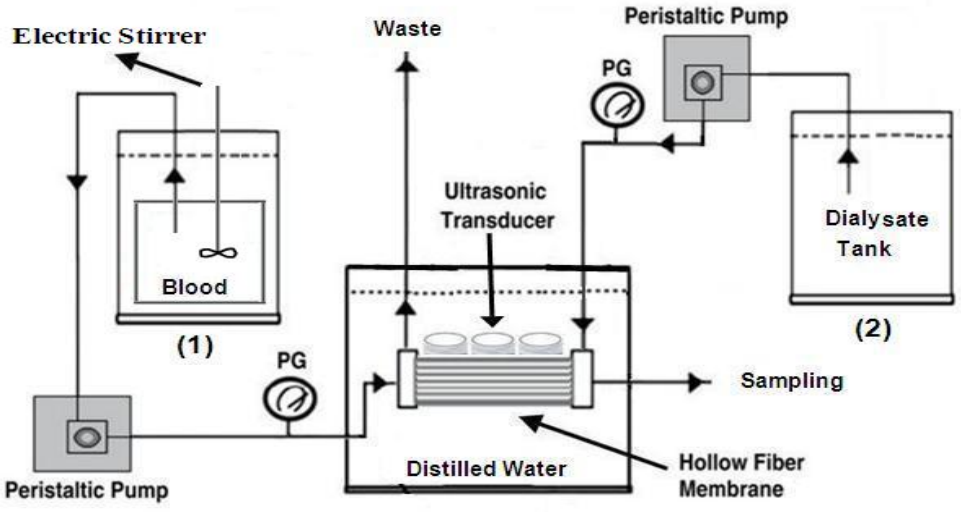

Figure 1: Schematic diagram of the experimental set up.

equation, the clearance (CL) of any substance was calculated [24-26]:

$$CL = Q_{Bin} (1- C_{Bout} / C_{Bin}) \quad (1)$$

Where Q_{Bin} = 200 (ml/min) is the flow rate of blood, C_{Bin} and C_{Bout} are respectively concentration of blood that input to the dialyzer (t=0) and outlet of the dialyzer for every 5 minutes.

Statistical Analysis

SPSS package (SPSS Inc., Chicago, IL, USA) for Windows® version 16.0 was used to analyze the data. The generation of graphs was performed using the Microsoft Office Excel 2013. Data were analyzed for statistical differences ($p < 0.05$) using one-way ANOVA.

Results

The clearance of each substance was calculated in the presence and absence of ultrasound and the results were compared. Results of the effect of ultrasound on Creatinine clearance are shown in Figure 2. As shown, ultrasound

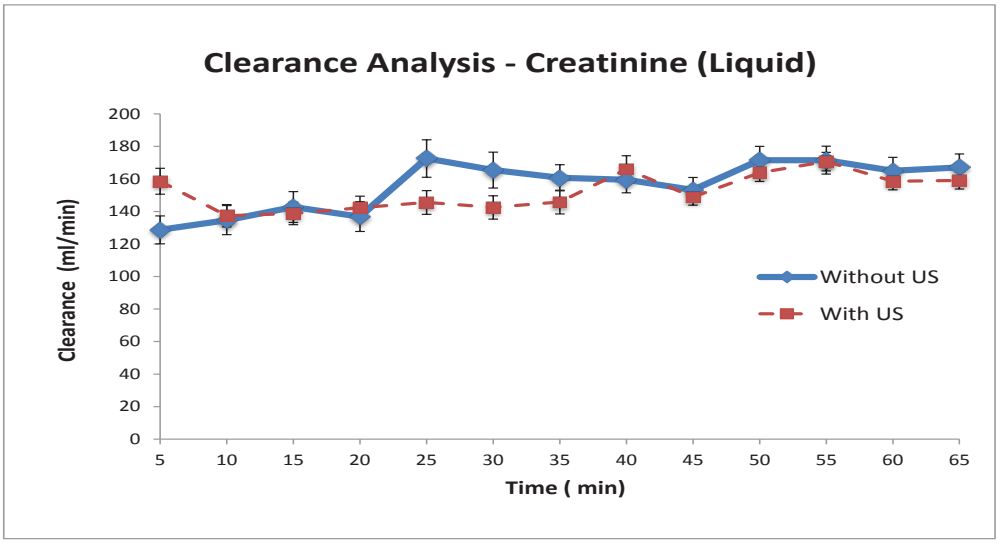

Figure 2: Effect of ultrasound on Creatinine clearance from blood equivalent solution

has no significant effects on the clearance of Creatinine from blood equivalent solution. The alteration of Urea clearance from blood equivalent solution is depicted in Figure 3, where a significant effect on the rate of clearance is observed in the presence of ultrasound. Our experiments on blood equivalent solution were followd by evaluating how ultrasound affects the clearance of Inulin (Figure 4). We observed that ultrasound significantly increases the amount of cleared Inulin. To make a good comparison, we calculated the mean of clearance of Creatinine, Urea and Inulin from blood equivalent solution versus time in the presence and absence of ultrasound as shown in Figure 5.

In the seconed part of this study, the effect of ultrasound on Creatinine clearance from human whole blood is shown in Figure 6. As indicated in this Figure, ultrasound has no significant effects on the clearance of Creatinine from whole blood, therefore, a correlation be-

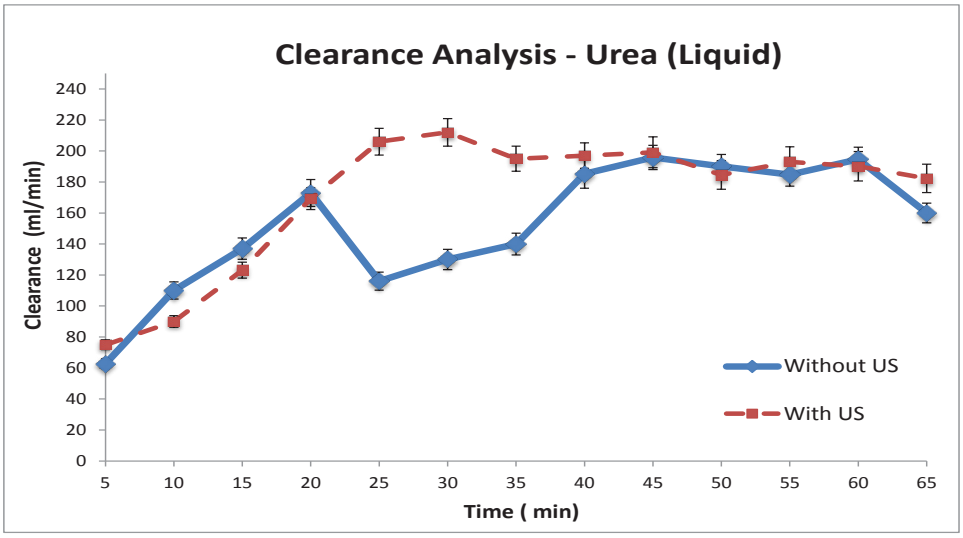

Figure 3: Effect of ultrasound on Urea clearance from blood equivalent solution

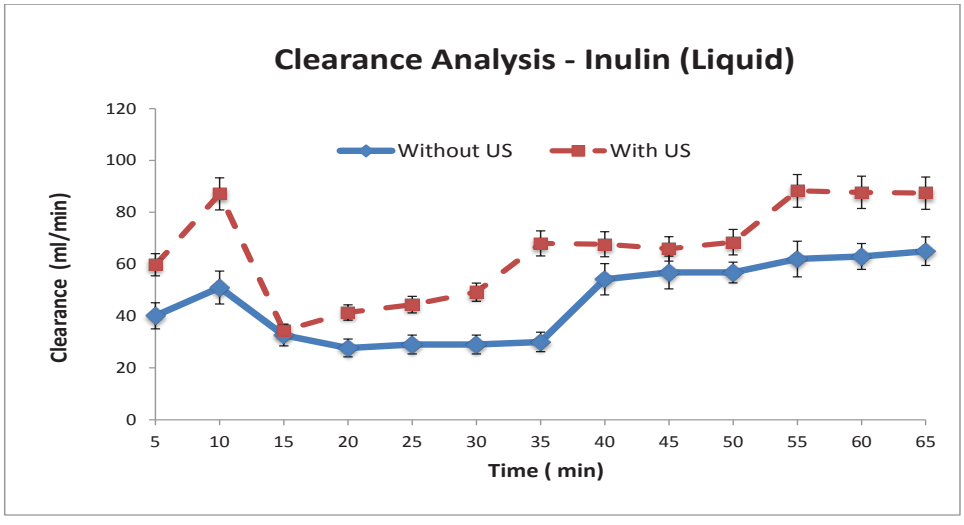

Figure 4: Effect of ultrasound on Inulin clearance from blood equivalent solution

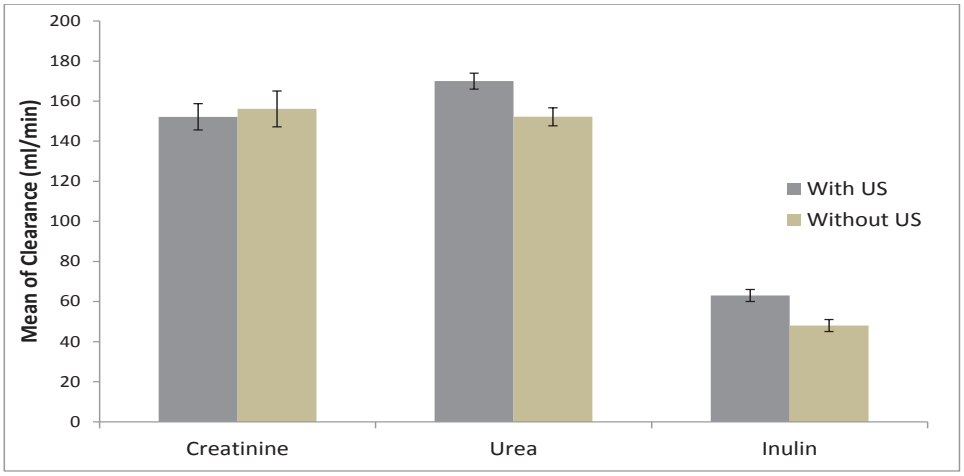

Figure 5: The mean of clearance of Creatinine, Urea, and Inulin from blood equivalent solution versus time in the presence and absence of ultrasound.

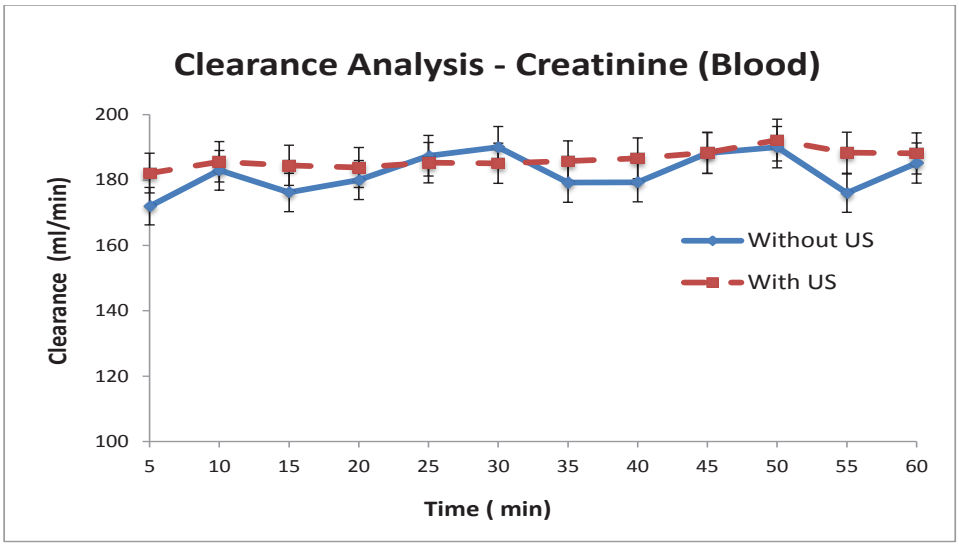

Figure 6: Effect of ultrasound on Creatinine clearance from blood

tween whole blood and the equivalent solution exists. Changes in Urea clearance from whole blood is shown in Figure 7, where a significant effect on the rate of clearance is observed in the presence of ultrasound waves. The clearance of inulin from whole blood is shown in Figure 8. We observed that ultrasound significantly increases the amount of Inulin cleared from the whole blood. Figure 9 presents a good comparison of the mean of clearance of Creatinine, Urea and Inulin from blood versus time in the presence and absence of ultrasound.

Discussion

The improvement of filtration and clearance with the aid of ultrasound have been so far reported by different researchers. Li et al. [22] showed that the permeate flux of clay solution in hollow fiber ultrafiltration membrane increased up to 95% in the presence of 40 kHz ultrasound. Researchers reported that the application of ultrasound at a frequency of 500

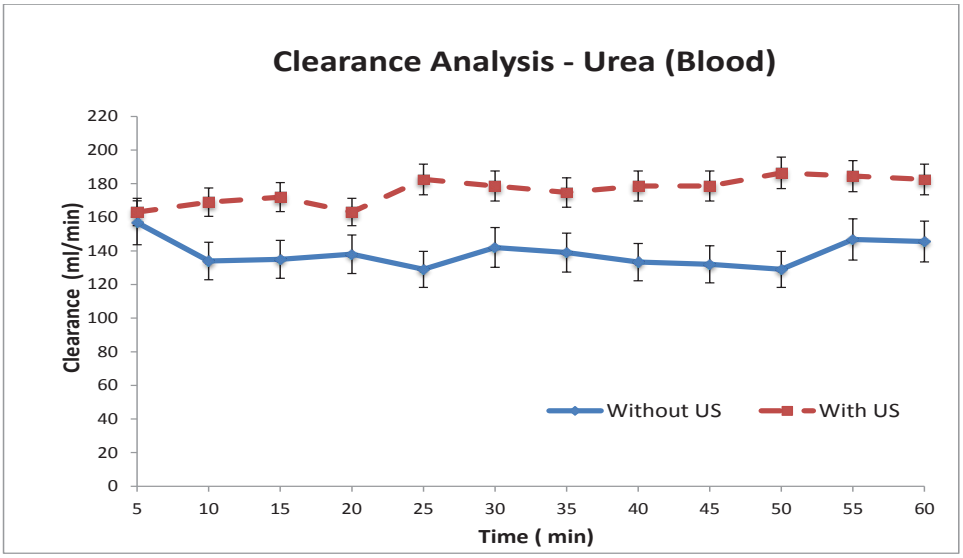

Figure 7: Effect of ultrasound on Urea clearance from blood

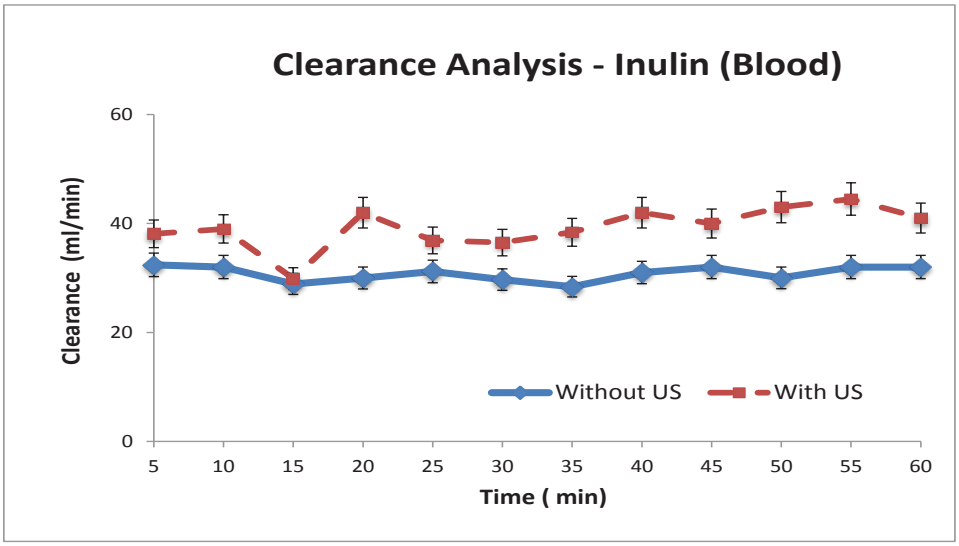

Figure 8: Effect of ultrasound on Inulin clearance from blood

kHz in dialysis membrane had a significant enhancement by 28% on steady-state permeation rate [14]. They used sodium acetate and sodium propionate as feed solutions. Wei et al. [17] investigated the effect of ultrasound cleaning on polluted polyvinylidenefluoride hollow fiber ultrafiltration membrane. They illustrated that permeate flux increased to 81% when membrane was cleaned with 2 gL^{-1} diluted citric acid in the presence of ultrasound.

Tatsumoto et al. [22] applied an aqueous solution of creatinine instead of blood in polyvinylchloride hollow fiber tubes and reported that creatinine clearance was improved approximately 1.5 times by 41 kHz ultrasound.

In this study, the effect of ultrasound on PES High Flux 130 Dialyzer with hollow fiber membrane that is commercially available was tested.

Statistical analysis indicated that pulsed

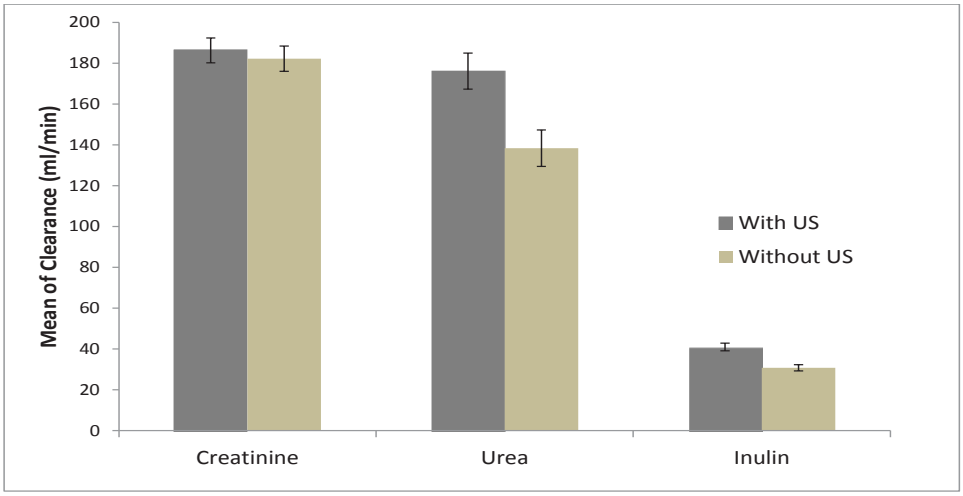

Figure 9: The mean of clearance of Creatinine, Urea, and Inulin from blood versus time in the presence and absence of ultrasound.

ultrasound did not have any significant effect ($p<0.05$) on the clearance of Creatinine. However, ultrasound did have significant effect ($p<0.05$) on Urea and Inulin clearances from blood. The results show that ultrasound irradiation of dialyzer increases the clearance of Urea from blood equivalent solution and whole blood by 11.7% and 27.3%, respectively. Moreover, it was confirmed that ultrasound irradiation of dialyzer increases the clearance of Inulin from blood equivalent solution and whole blood by 31.3% and 33.1%, respectively. The molecular weights of Urea and Creatinine (60.06 and 113.12 Da) are very small, and they should pass through pores of hollow fiber membrane easily. Ultrasonic irradiation, therefore, should not affect the clearance of these two substances; however, the clearance of Urea is still enhanced in the present study. This is probably due to the particular structure of the blood in our study which is not compatible with previous studies. In the case of Inulin, which is a medium molecular weight substance (5200 Da), fouling of pores occurs in the membrane, and ultrasound significantly reduced this fouling. Results obtained for Urea and Inulin express that using pulsed ultrasonound waves with a frequency of 1 MHz and intensity of 1 W/cm^2 can cause cleaning of hollow fiber membrane and hence prevent the reduction of filtration.

In an attempt to mitigate the fouling in hollow fiber membrane of High Flux dialyzer, pulsed ultrasound irradiation with frequency of 1 MHz at an intensity of 1 W/cm^2 was applied. We reported significant improvements on clearances of Urea and Inulin in High Flux PES 130 Dialyzer with Polyethersulfone hollow fiber membrane. Considering the capabilities of ultrasound and what reported in this study, there is a great potential to enhance the eficacy of routin hemodialysis which is accomplished in clinic.

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Conflict of Interest

None

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