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## Analysis of Blood Purification Studies on Oxone Mediated TEMPO-Oxidized Nano Cellulose Mixed-Matrix Membranes

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**Analysis of Blood Purification Studies on Oxone Mediated TEMPO-Oxidized Nano  
Cellulose Mixed-Matrix Membranes**

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University of Arkansas

**Abstract**

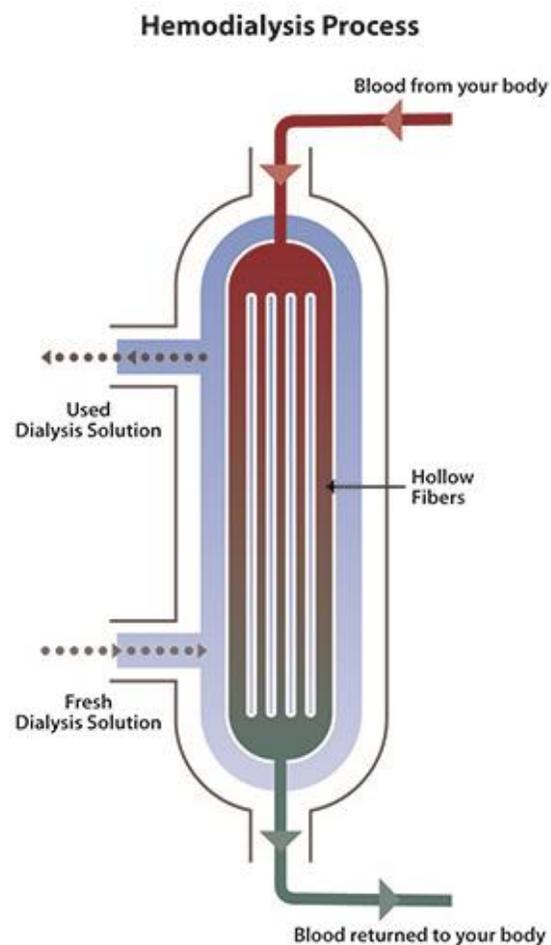
End-stage renal disease (ESRD) is currently the ninth leading cause of death in the United States, and of the 661,00 Americans diagnosed with ESRD, approximately 468,800 were on hemodialysis in 2016. Hemodialysis refers to a technique where a machine combined with a membrane, often referred to as an artificial kidney, is used to clean blood by removing any waste such as urea, potassium, and other smaller waste products while preserving the concentrations and integrity of cells and proteins in the blood. It has been shown in artificial blood studies that cellulose nanomaterials, like TEMPO/Oxidized cellulose nanoparticles (TOCNs), can be integrated into membranes that subsequently display desirable membrane and membrane transport properties such as increased flux, urea clearance and fouling resistance. The focus of this study was on the application of three variants of these hollow fiber mixed-matrix membranes that were derived from two types (partially/fully oxidized) of TOCNs. Hemodialysis was performed using hematocrit adjusted bovine blood to analyze a number of transport properties of each membrane including protein rejection, blood cell rejection, ionic permeability, and urea clearance. The 50/50 mixed-matrix membrane showed promising results with its high ionic permeability to divalent cations and improved urea clearance and theoretical treatment time all while retaining a comparable concentration of erythrocytes, leukocytes, thrombocytes, and blood proteins. Form I and Form II both rejected blood cells and proteins similar to 50/50, however, they both had a reduced permeability to divalent cations while also showing little to no improvement in urea clearance or treatment time when compared to the control. For these reasons, the 50/50 membrane was distinguished as the most viable membrane to base future blood hemolysis and membrane characterization studies around due to its exceptional urea clearance, reduction of theoretical treatment time, and desirable ionic permeability properties.

## 1. Introduction

According to the Center for Disease Control and Prevention, it is estimated that more than 1 in 7 US adults currently have chronic kidney disease (CKD)[1]. CKD occurs when an individual's kidneys become damaged and lose their ability to adequately filter their blood. If left untreated, CKD has the possibility to progress to further health complications such as kidney failure, also known as end-stage renal disease (ESRD), which is currently the ninth leading cause of death in the United States [1]. The two main treatments for ESRD are dialysis or a kidney transplant, however, according to the National Institute of Diabetes and Digestive and Kidney Diseases, of the approximately 661,000 Americans diagnosed with ESRD, 468,000 were on dialysis while only 193,000 were living with transplants in 2016 [2]. Kidney transplants have been shown to provide an improved quality of life and reduced risk of mortality over dialysis, however, the most recent available statistics from the U.S. Department of Health and Human Services shows that there were 92,194 Americans on the waitlist for a kidney transplant at the end of 2018, and 40% of individuals that were on the waitlist in 2015 were still on the waitlist in 2018 [3][4].

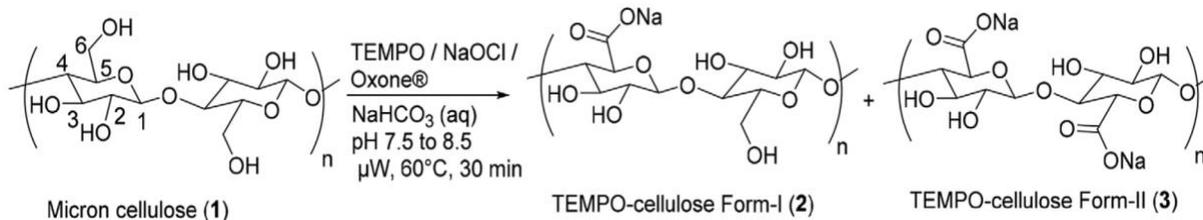
While individuals suffering from ESRD might wait years for a kidney transplant, there is a clear need to make improvements upon the current dialysis treatments that are relatively widely available when compared to eligible kidney donors that match. One of the main types of dialysis that patients with ESRD will undergo is hemodialysis. Hemodialysis refers to a technique where a machine combined with a membrane is used to clean blood by removing any waste such as urea, potassium, and other smaller waste products. A dialysate solution is used as a washing fluid to create an osmotic and chemical gradient for the waste in the blood to make an exchange across

the semipermeable membrane by simple diffusion. By changing the ionic concentrations and composition of the dialysate, a hemodialysis machine can be finely tuned to select for which ions of the blood should be removed or added. The membrane is often referred to as a dialyzer or artificial kidney since it is the component of the hemodialysis machine which filters and has direct contact with the patient's blood. The dialyzers are hollow fiber membranes that allow blood to pass through the center of the straw-like cylinders while the dialysate solution is pumped countercurrent to the direction of the blood flow within the hollow fiber membranes. Figure 1 below provides a representation of a simplified hemodialysis machine.



**Figure 1:** A hemodialysis hollow fiber module. [5]

There are a number of high performance dialyzers that are currently on the market which are made from synthetic materials such as polysulfone (PSf) or cellulose triacetate (CTA). These materials are favored for use in hemodialysis due to their excellent biocompatibility and ability to effectively clear target solutes with pore sizes larger than most conventional hemodialysis membranes [6]. Since cellulose is the most abundant natural polymer on Earth, it is a very appealing material to work with because it is relatively inexpensive and easy to handle. It is also well documented that cellulose membranes can be modified to improve their performance during hemodialysis thus decreasing the mortality rate when compared to other unmodified membranes [7]. Through the use of cellulose nanomaterials (CNM), these existing CTA membranes can be further modified to improve a multitude of membrane properties such as membrane strength and hydrophilicity, and transport properties which could consequently improve membrane separation [8]. Preliminary research on CNM modifications on cellulose membranes, specifically the Form I, Form II, and 50/50 hollow fiber Oxone mediated TEMPO-oxidized nano cellulose mixed-matrix membranes produced by Kristyn Robling in her 2019 thesis, have shown promising membrane properties such as increases in flux, mass transfer area coefficient, urea clearance, and antifouling properties [9]. TEMPO-cellulose Form I is partially oxidized while TEMPO-cellulose Form II is fully oxidized. The chemical structure and recently developed production method of these two derivatives can be seen in Figure 2 below.



**Figure 2:** The production method from micron cellulose in microwave irradiation conditions of Form I and Form II, and the both derivatives chemical structures. [10]

In collaboration with the University of Arkansas for Medical Sciences, and continuing the work done by Kristyn Robling, it is the goal of this research to further characterize the Oxone mediated TEMPO-oxidized nano cellulose mixed-matrix membranes through a series of *in vitro* hemodialysis studies using hematocrit adjusted bovine blood. It is hypothesized that the derivatives Form I will show an increases in urea clearance and ion permeability, and will display comparable biocompatible properties to the control, CTA membranes.

## 2. Materials and Method

### 2.1 Materials

Assembled modules each containing 20 fibers of a designated type, CTA, Form I, Form II, 50/50, were previously assembled following the procedures outlined in Robling's 2019 thesis. These hollow fiber membranes were created using CTA from Millipore Sigma, N-methylpyrrolidone (NMP), and deionized water along with pure TEMPO/Oxone-oxidized cellulose nanocrystals (TOCNs) and Form I and Form II samples that were prepared by Dr. Peter A. Crooks from the University of Arkansas for Medical Sciences. The membranes were developed according to the proportions laid out in Table 1 below.

**Table 1:** Composition by weight percentage that the three modified membranes were composed according to. [9]

Membrane	TOCN Form I (wt.%)	TOCN Form II (wt.%)	CTA (wt.%)	NMP (wt.%)
(a) Form I	1.0	0.0	9.0	90.0
(b) 50/50	0.5	0.5	9.0	90.0
(c) Form II	0.0	1.0	9.0	90.0

Hematocrit adjusted bovine blood from Lampire Biological Labs was used as a model for human blood throughout the experiments. A fluid pump, dialysate (PBS) solution, warm water bath, pressure-rated heparinized dialysis tubing, and beakers to receive the feed out and dialysate out were required to begin experimental setup. A 70% ethanol solution would be frequently required to sterilize the station, and proper personal protective equipment was necessary as these experiments would be with animal blood.

## 2.2 Hemodialysis

To prepare for hemodialysis experiments, the modules were activated through a membrane conditioning step. This step is crucial because it essentially primes the membranes for the remainder of the experiment by allowing them to adjust to the aqueous pressurized environment. The flux of these membranes will vary slightly over time shortly after being exposed to a solution. To condition the modules, they were loaded into the apparatus in such a way that the eventual dialysate would run countercurrent to the feed bovine blood that would travel through the 20 hollow fibers, however, the membranes were initially activated using 25 °C deionized water with a consistent transmembrane pressure of 3 psig for one hour. The deionized water was passed through the dialysate and feed space both inside and outside of the hollow

fibers. Once the membranes reached a more stable state where the pure water flux was changing less than 5% per minute, then the blood studies would be initiated. The feed was run at a flow rate of 300 mL/min and the dialysate was run at a flow rate of 500 mL/min.

While the membranes were being conditioned, the feed bovine blood was warmed in a bath to a temperature of 37 °C. When stabilization was reached, the necessary connections were switched to facilitate hemodialysis, which involved removing the deionized water feed connections and connecting warmed bovine blood to the feed in. The blood was allowed to clean run for a few minutes to clear out the residual water that was left in the system. Modules were then run in parallel in the interest of time for thirty minutes making sure to collect the used dialysate and feed blood. Samples were then taken to be analyzed later. Upon completion of the first two modules, these modules were switched out to run the remaining two.

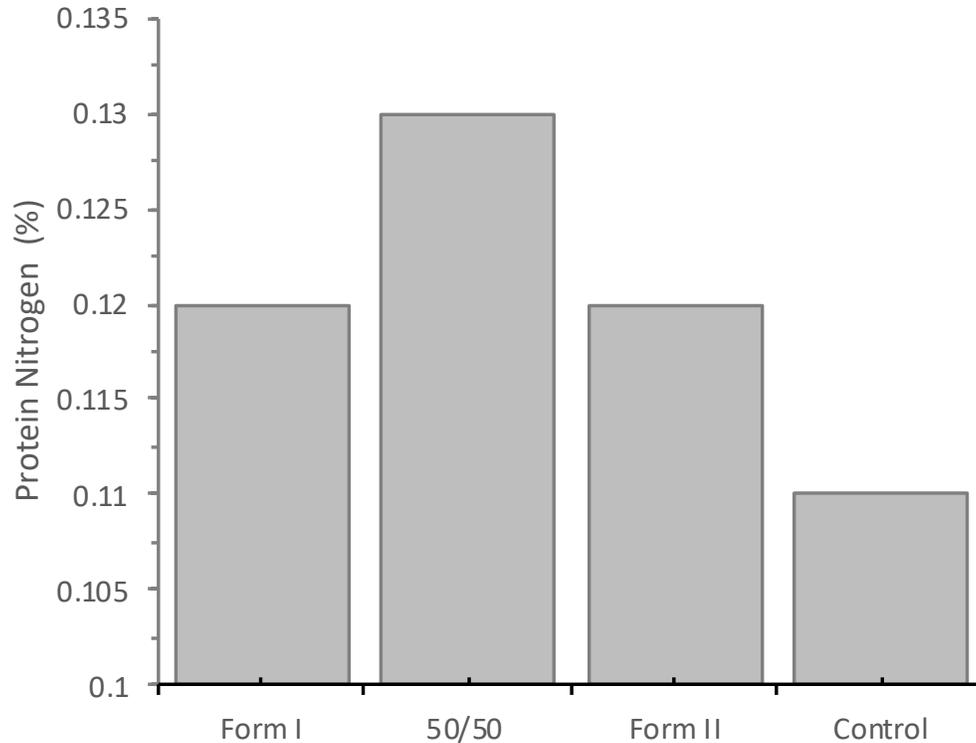
When all samples were collected, the hemodialysis was shut down and the module was disconnected. A 70% ethanol/water mixture was run through the apparatus without the modules attached to disinfect it. All materials were properly sanitized and disposed of in the proper bins.

### **2.3 Sample Testing**

The samples collected during hemodialysis were tested using a 96-well plate reading UV-vis spectrophotometer with a colorimetric assay for urea concentration. For protein analysis, samples were sent off to the Central Analytical Laboratory in Poultry Science Center at University of Arkansas where serum protein quantity was analyzed by free nitrogen adsorption method. Blood analysis was performed in poultry science on the multi-animal blood analyzer. Lastly, salt concentration was analyzed using inductive coupled plasma ISP.

### 3. Results

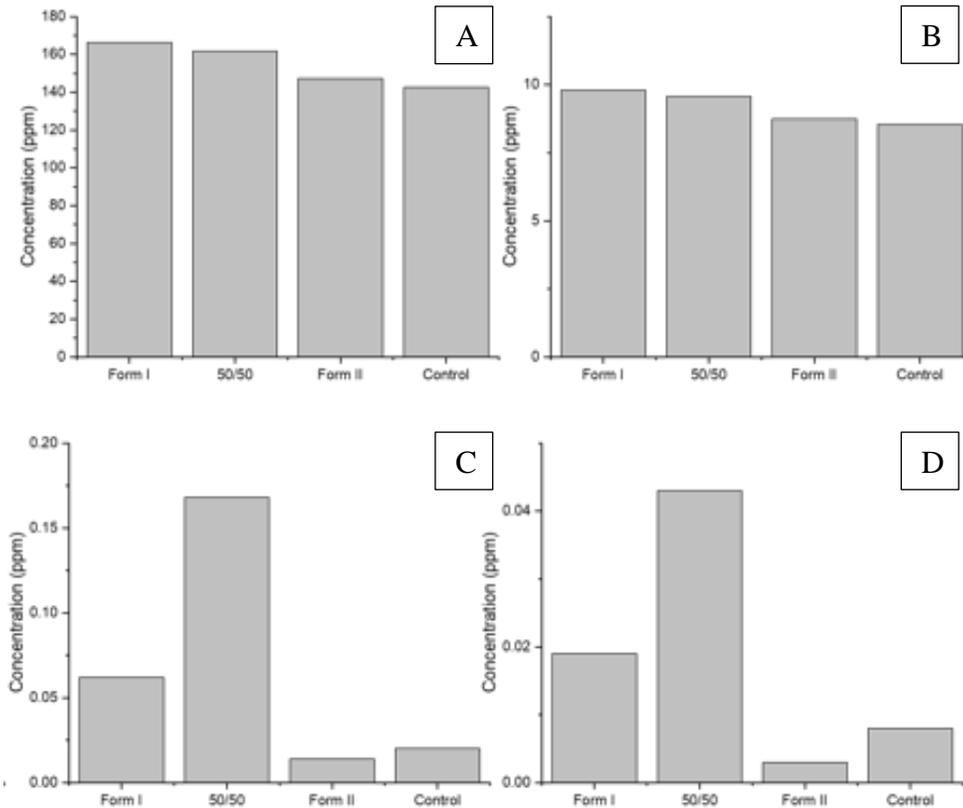
#### 3.1 Protein Analysis



**Figure 3:** Protein concentrations measured for the dialysate solutions that are used for the corresponding membrane type.

Serum protein quantity that was analyzed by free nitrogen adsorption method showed that all membranes performed essentially the same with the biggest difference of 0.02% in dialysate protein concentration being between the 50/50 and control dialysates. This suggests that all three TOCN membranes reject blood proteins at a similar rate as the CTA control.

### 3.2 Ionic Transport Characterization



**Figure 4:** Varying ion concentrations measured for the dialysate solutions that are used for the corresponding membrane type. Ions tested included: (A) Na<sup>+</sup>, (B) K<sup>+</sup>, (C) Mg<sup>2+</sup>, (D) Ca<sup>2+</sup>.

All modules performed similarly for allowing the monovalent cations, sodium and potassium, to pass through the membranes. However, when it came to the divalent cations, magnesium and calcium, the 50/50 membrane seemed to be much more permeable than the control, Form I, or Form II. A higher ion permeability would suggest that hemodialysis could be more easily controlled by close monitoring of patient blood ion levels and necessary adjustment of dialysate solution composition.

### 3.3 Blood Transport Characterization

**Table 2:** The blood transport characteristics of cellulose dialysis membranes.

Sample	Platelet Rejection %	White Blood Cells K/uL	Red Blood Cells M/uL	Hemoglobin Concentration g/dL	Platelets K/mL
Initial Blood	N/A	10.36	7.33	10.1	447
Form I	0.98	0.02	0	0	7
50/50	0.98	0.02	0	0	11
Form II	0.98	0.02	0	0	7
Control	0.98	0.02	0	0	9

All three mixed-matrix membranes showed the ability to reject erythrocytes, leukocytes, and thrombocytes in the blood as efficiently as the control. Platelet counts in the permeates were comparable to the control. These results suggest that all tested mixed-matrix membranes have pore sizes that are small enough to prevent important blood components, which are retained within the kidneys in normal renal filtration, from leaking into the permeate and causing the blood to become anemic.

### 3.4 Urea Transport Characterization

**Table 3:** The urea transport characteristics of cellulose dialysis membranes.

Membrane Sample	$K_{oA}$	Experimental Clearance (mL/min)	Theoretical Clearance for 1 m <sup>2</sup> membrane (mL/min)	Theoretical time to reach kt/V of 1.2 (minutes)
Form I	685	7.7	237	213
50/50	1261	11.9	275	183
Form II	888	9.2	255	198
CTA	842	10.1	251	200

The  $K_{oA}$  is the mass transfer area coefficient. The mass transfer area coefficient is a number that can provide a theoretical clearance value when there is infinite blood and dialysate flow rates.  $A$  was membrane surface area for each module. Fick's law (Equation 1 below) was

used to calculate the mass transfer area coefficients for each module tested. The solute transport rate,  $N$ , was calculated using Equation 2.  $\Delta C_M$  was the log mean concentration difference and was calculated using Equation 3 below. The experimental clearance,  $K_{Experimental}$ , was calculated using Equation 4 below. The mass transfer coefficient and experimental urea clearance values for each module tested are in Table 3 above.

$$\text{Equation 1: } N = K_{oA}A(\Delta C_M)$$

$$\text{Equation 2: } N = Q_f(C_{fi} - C_{fo}) = Q_d(C_{do} - C_{di})$$

$$\text{Equation 3: } \Delta C_M = \frac{(C_{fi} - C_{do}) - (C_{fo} - C_{di})}{\ln\left(\frac{C_{fi} - C_{do}}{C_{fo} - C_{di}}\right)}$$

$$\text{Equation 4: } K = \frac{N}{(C_{fo} - C_{di})}$$

The  $K_{Experimental}$ , log mean concentration difference, mass balance, and Fick's law were all used to solve for the theoretical clearance for a membrane with a surface area of 1 m<sup>2</sup>. This solved equation (Equation 5) can be found below where  $Q_f$  is equal to the flow of the feed blood and  $Z$  is the blood flow rate divided by the dialysate flow rate.  $R$  is equal to the mass transfer coefficient multiplied by the theoretical membrane area, which in this case is 1 m<sup>2</sup>, divided by the flow rate of the dialysate.

$$\text{Equation 5: } K = Q_f \frac{1 - \exp(R(1-Z))}{Z - \exp(R(1-Z))}$$

The theoretical clearance of a 1 m<sup>2</sup> membrane was then used to calculate the theoretical treatment time. A model person with a mass of 70 kg and a 60% water weight was used to calculate the theoretical treatment time for them to reach a Kt/V of 1.2, which corresponds to the Kt/V target set by the US National Kidney Foundation. Results showed that the 50/50 membrane

reduced the treatment time by the greatest amount. Form II had a similar treatment time to the control while Form I had a higher theoretical treatment time than the control.

#### **4. Conclusion**

After analysis of each membrane, there were improvements found for the 50/50 module over the control (CTA) high performance membrane when it came to divalent cation permeability and urea clearance while also performing similarly to the control with regards to rejection of major erythrocytes, leukocytes, thrombocytes and blood. All three mixed-matrix membranes were slightly more permeable to monovalent cations than the control, and all three proved to reject proteins and cells in the blood at a comparable level as the CTA control membranes. When determining theoretical treatment times, 50/50 was found to have largest mass transfer coefficient and the shortest treatment time compared to the control. Although it was predicted that the Form I module would perform best during hemodialysis, it appears that the 50/50 module had the best performance and is a promising candidate for further hemodialysis testing of itself and varying derivatives of mixtures of Form I and Form II in the future.

#### **5. Future Work**

The next step in experiments with these membranes with regards to hemodialysis should involve developing new membranes of varying ratios to form a clearer picture of the interaction of the two CNMs in the membranes. Given that these CNM mixed-matrix membranes displayed biocompatible properties throughout this research and have previously shown low-fouling properties, the potential for the eventual development of an implantable artificial kidney device should be investigated as current technology progresses.

## **6. Acknowledgements**

Special thanks to my thesis advisor, Dr. Jamie Hestekin for his committed guidance and assistance throughout this thesis project. I would also like to recognize and show my appreciation to both John Moore and Efecan Pakkaner, University of Arkansas Chemical Engineering Graduate students, for their enthusiastic assistance and unwavering commitment to guide me throughout the course of this research.

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