Introducing Biomedical Engineering Using Creatinine Based Time-in-Dialysis Experiment

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In the emerging field of biomedical engineering, there is a need for experiments which can illustrate the importance of engineering concepts in medicine. One of the laboratory exercise used in demonstrating the fundamental concepts is hemodialysis device. Typically it is used under simulated conditions via salt solutions with the focus of providing hands-on experience on separation concepts.

At OSU, we extended the experiment to a more clinically relevant project by using creatinine to represent blood toxins; the function of the kidneys is measured clinically by determining the clearance rate of creatinine, a metabolic by-product of the muscles that remains fairly constant. Thus we asked the students to evaluate the clearance rate of creatinine in a commercially available hemodialyzer, and model the system; pure water represented blood. They were told to monitor the pressure difference across the membrane in the removal of water from blood and also to alter the flow rates. A picric acid based spectroscopy was used to monitor the changes in creatinine concentrations in the blood.

The "patient" was modeled as a Continuous Stirred Tank with a known initial creatinine concentration, and sodium chloride concentration. Furthermore, the dialysate osmotic pressure was altered by introducing sugar. Using concepts of compartmental modeling and shell-and-tube heat exchanger, differential equations were developed. These equations were solved using a math solver, POLYMATH, to predict the outlet blood concentration, the amount of excess water removed, and the required time-in-dialysis given initial blood conditions and machine operating conditions. This multi-level experiment not only reinforces the engineering concepts and physiology but also sound mathematical approach to problem solving. Implications of this experience will be discussed in detail.

Introduction

The recent boom in biomedical and biotechnology programs has necessitated new bio-based experiments in the engineering curriculum. At Oklahoma State University, two new courses have been developed in the School of Chemical Engineering to integrate the biological concepts. These two courses a) Introduction to Biomedical Engineering and b) Bioprocess Engineering are offered as electives for students in the senior year. In addition, to provide hands-on experience with the few concepts discussed in each course, two new experiments a) bioreactor design for the conversion of renewable resources and b) dialysis experiment for the clearance of creatinine, have been added into the second Unit Operations Laboratory (UOL) offered in the Fall of the senior year. In tandem with this course, students are enrolled in an optional "Introduction to Biomedical Engineering" course.

In UOL, students work in teams of three, on three different projects. Teams are assigned by the instructors and care is taken to avoid repetition of members in more than one project. While assigning the project, the bio-related projects are allocated preferentially to the students either enrolled in the biomedical course or committed to the Bioprocess Engineering courses. Each project covers a 5-6-week period. It includes a week of planning which has to be approved by the instructor prior to students' working in the laboratory. This is followed by three 6-hr

laboratory sessions. Prior to second and third laboratory sessions students update the instructor about the progress on the project either through a memorandum or through oral discussions. To ensure that students include all issues, and properly analyze the data, instructors actively observe and coach the teams as they work. After the laboratory sessions, students present their work to the rest of the students and other instructors. During the presentation or after the presentation, students will be given feedback about the presentation and necessary changes to be incorporated in their final report due a week later. Each activity is graded including the preplan, progress reports, oral presentation and written report. One of the laboratory exercises used in demonstrating the fundamental concepts is a hemodialysis device. Typically, it is used under simulated conditions via salt solutions with the focus of providing hands-on experience on separation concepts [1]. At OSU, we extended the experiment to a more clinically relevant project by using creatinine to represent blood toxins. The project is described below.

Project Statement.

Our company makes biomedical devices, one of which is a hollow-fiber separator for dialysis machines. Hospitals use blood dialysis to process the blood of patients whose kidneys cannot remove small-molecule protein waste from the blood. The machine has many features that you will not need to use. We are interested in the hollow-fiber membrane separator-the purification device. Please develop and validate from first-principles a dynamic model (non-steady state, transient) of the separation of "poison proteins" (creatinine in our experiment) and water from "blood" (water in our experiment) in a patient.

Generate experimental data from the hollow-fiber membrane separator to obtain an overall transfer coefficient for creatinine and water transfer, and validate your model. The hollow-fiber membrane unit is similar to a shell-and-tube heat exchanger. Basically, blood flows through the inside of the hollow fibers (tubes), and dialysate (clean water) flows through the shell side. Unwanted proteins then diffuse through the fiber walls from the concentrated tube fluid to the dilute shell fluid. It appears that the separation mechanism is analogous to heat transfer in a fluid-fluid shell-and-tube exchanger, but with mass transferring through the wall instead of heat. However, there is a complication. Since kidneys also remove water from the blood, and since dysfunctional kidneys do not; in the hospital, pressure differences on the tube and shell sides, enhanced by osmotic pressure differences when sugar water is used in the shell-side, draws water out of the blood. Another concern during dialysis is the alterations of electrolyte concentrations in the blood. Use NaCl as the electrolyte and monitor the changes using conductivity. You will observe that the pressure drop between the "blood" and "dialysate" causes the water to migrate through the fiber wall. Usually the blood pressure is high enough to cause a net flow of water from the tube-side to the shell-side of the separator.

The patient's blood is continuously circulating within his/her body. The blood compartment of the body can be assumed as a Continuous Stirred Tank (CST) in which a small portion is partially purified, and returned from the kidney dialysis. Typically, dialysis is performed every other day and lasts for two hours. As dialysis proceeds, the waste content diminishes in the patient, and the rate at which the hollow-fiber separator removes the waste decreases. Using your model to determine the best combination of time-in-dialysis, blood recirculation rate, and Trans-membrane pressure (TMP) for the patient to be 90% purified of poison and 100% relieved of excess water. You should be prepared to present your work to the technical support group on the XX-DAY. The final written report submission deadline is on or before XX-DAY+7.

Experimental Setup.

The experiment consisted of a hemodialysis machine (a donation from a local dialysis center, shown in **Figure 1A**). At the inlet and outlet of the dialyzer, long tubes were mounted vertically and used as monometers. Five-liter solution of 7.5 mM creatinine and 140 mM NaCl was used to simulate the patient's initial blood concentration. The solution was continuously mixed during the experimental period using a magnetic stirrer. The inlet and outlet surgical tubes were placed into the bucket and the machine was started. The blood was pumped through the machine at 300mL/min and blood volume changes were monitored at regular intervals by weighing on an electronic scale. TMP, flow rate of fluids was regulated using the controls on the machine.

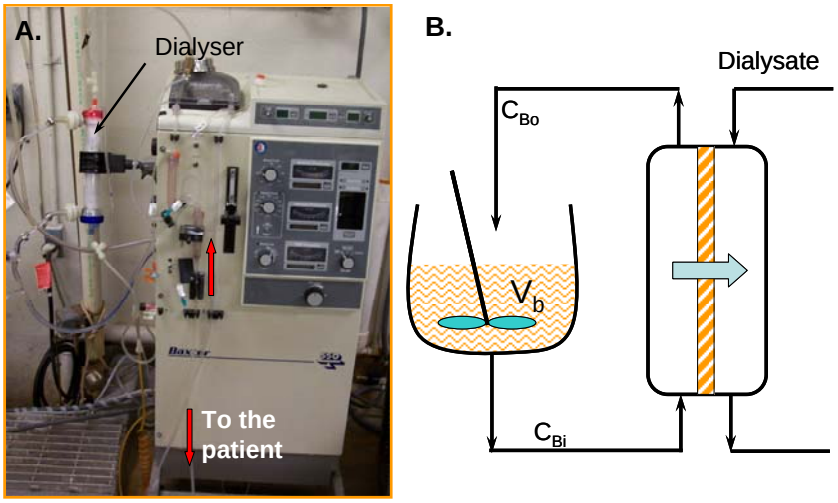

Figure 1: Panel A. Equipment used in the project. **Panel B.** Process schematic**.**

Experiments were conducted over a two hour period in two ways i) open loop to determine the transport parameter, and ii) closed loop to determine the effectiveness of the dialysis. At five-minute intervals, the inlet and outlet pressures across the dialyzer, the weight of the patient and conductivity of the solution were recorded. In addition, 0.3-0.5mL samples were collected for analysis of the creatinine concentration. The weight of the patient (bucket) was converted to the change in volume as a function of time.

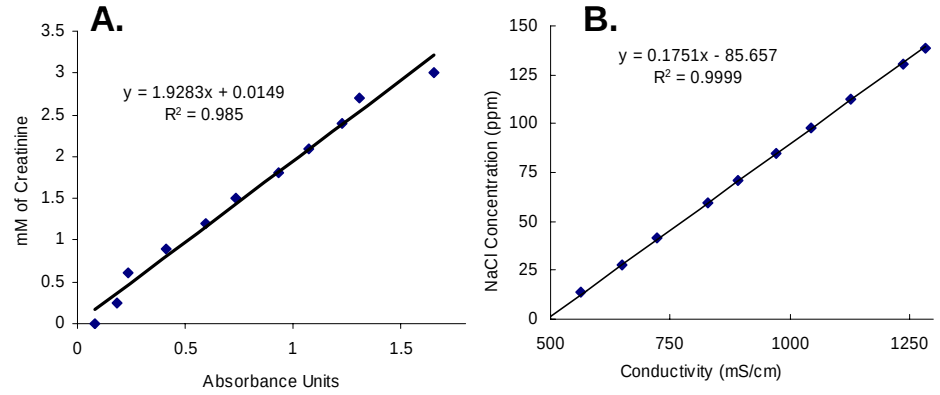

Figure 2. Calibration plots for Creatinine (Panel A) and NaCl (Panel B).

To analyze the creatinine concentration, samples were mixed 1:3 ratios with solution containing 10:1 ratio of 0.14% picric acid and sodium hydroxide. Note that picric acid is hazardous, highly

explosive and should be used under the carefully guidance of an instructor or a safety personnel. These mixtures are then placed into a spectrophotometer and the absorbance is measured at 490 nm. The concentrations were determined using a correlation equation obtained using known concentrations of creatinine. A similar correlation was obtained for conductivity measurements as shown in **Figure 2**. An equation for the line was generated by linear regression in Microsoft Excel. Students were already aware of the regression coefficient function as it was in other courses. Obtained concentration values were plotted as a function of time (**Figure 3**).

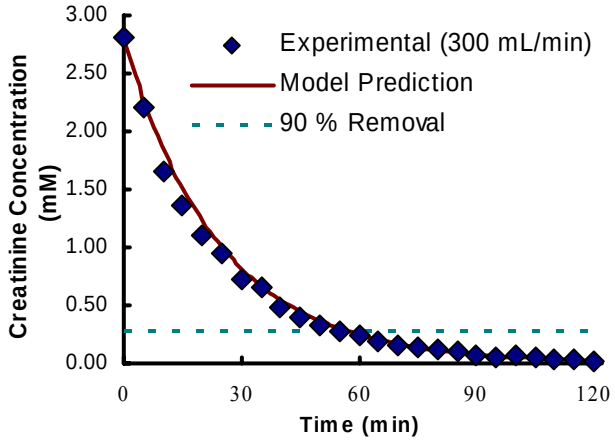

Figure 3. Concentration as a function of time.

Modeling the Process.

To model the observed results, compartmental modeling was used [2] and the patient was assumed as a CST. The flow diagram shown in **Figure 1B** was used. From the basics of chemical reactor design, the governing material balance is

$$Q \cdot C_{Bo} - Q \cdot C_{Bi} = \frac{dV_B C_{Bi}}{dt} \qquad (1)$$

where C_{Bo} is the concentration of creatinine in blood, entering patient out of dialyzer (g/mL), C_{Bi} is the concentration of creatinine in blood, leaving patient into dialyzer (g/mL), Q is the volumetric flow rate of blood (mL/min) and V_B is the volume of the blood. Since V_B and C_{Bi} are changing as a function of time (t), they need to be differentiated using changing rule. From the Starlings equation, the change in volume can be written as a function of the hydrostatic pressure difference and the osmotic pressure difference. In a simplified form

$$\frac{dV_B}{dt} = \alpha \cdot (\Delta P - \Delta \pi) \qquad (2)$$

where α is a proportionality constant in $\left(\frac{mL}{inHg \cdot \min} \right)$, Δπ is the osmotic pressure difference (in mmHg), and ΔP is average hydraulic pressure difference (inHg). The average hydraulic pressure difference can be calculated using

$$\Delta P = \frac{\left(P_{D,in} - P_{D,out}\right)}{2} - \frac{\left(P_{B,in} - P_{B,out}\right)}{2} \qquad (3)$$

where P_D is the pressure in dialysate (in Hg) and P_B is the pressure in "blood" (in Hg). The in and out are referenced from the point of the dialyzer [4]. In addition, osmotic pressures of blood can be calculated using

$$\pi = \frac{-R\,T}{V_w^L} \ln(\gamma_w^A\ x_w^A) \tag{4}$$

where R is universal gas constant $\left(\frac{in\,Hg \cdot mL}{mol \cdot {}^{o}R}\right)$, T is the temperature (^{o}R), γ_w^A = activity coefficient (unitless), x_w^A = mole fraction in solution (unitless), and V_w^L = molar volume (mL/mol). The activity coefficient of creatinine was assumed one, and the osmotic pressure for only sodium chloride/glucose was determined using the following equation. Since, both changes in volume and pressure (ΔP) are measured at various intervals, they can be plotted by neglecting changes in osmotic pressure. Using the slope, the proportionality constant can be obtained. Note that one could also correlate the obtained results with the TMP.

For a single pass through the dialyzer, equation 1 can be integrated assuming V_B constant to obtain

$$C_{Bo} = C_{Bi} \exp\left(\frac{-K\,A}{Q_B}\right) \tag{5}$$

where K is the transport parameter (m/min), A is the transport area (m^2), and Q_B is the volumetric flow rate of blood (m^3/min). Measuring the $C_{BO,}$ C_{Bi} and Q_B in an open loop arrangement, K values can be obtained using the A (1.5 m^2) value given by the manufacturer (Terumo Medical Corportation, Tokyo, Japan).

Combining Equations 1, 2 and 5, we get

$$\frac{dC_{B,in}}{dt} = \frac{C_{B,in}\,Q_B \exp\left(\frac{-K\,A}{Q_B}\right) + C_{B,in}[Q_B - \alpha(\Delta P - \pi)]}{V_B} \tag{6}$$

Equation 6 can be solved in a math solver such as Polymath to evaluate the creatinine concentration in the blood for the closed-loop experiment [3].

Results and Discussion.

Using an initial concentration of 0.0075 M, and final concentration of 0 M, model prediction is compared with the experimental creatinine concentration (**Figure 3**) as a function of time. For V_B one could plot the change in volume as a function of time MS Excel and a curve fit can be used. The K value was obtained to be 140 μm/min. The model predictions closely matched the experimental results suggesting that the usage of the model for other flow rates and concentrations. In addition, the time taken to remove 90% of creatinine can also be obtained which is close to 60 minutes. One could obtain a similar plot for NaCl and determine the loss of electrolyte concentration.

This experiment exposes students to a medical device such as the hemodialysis machine. Hands-on experience with this equipment allows for a deeper understanding of how the machine is operated and how the dialyzer functions. Deriving the differential equations from the continuity equation requires the student to draw on his or her math and engineering knowledge to create solutions. Students will use math solvers and Excel to solve the differential equations, formulate data spreadsheets and create charts. In addition, good laboratory techniques in millimolar range are developed and team problem solving skills are mastered. The hands-on learning achieved in the laboratory help students to connect the abstract concepts of the classroom with the real world.

Through an experiment of this type, undergraduates can integrate a number of concepts learned in the engineering curriculum and get a feel for the variety of aspects of biomedical engineering including transport processes, bioelectrical phenomena, osmotic pressure, protein assay, and modeling. The chief disadvantage of this method is the time requirement; this experiment will require four to six weeks. The long timeframe minimizes the number of experiments that may be performed. The project-oriented style will require a greater commitment from faculty members and less dependence on teaching assistants in the laboratory. However, short experiments tend to become modular and lack integration of comprehensive concepts. The advantage of this method is that it integrates concepts such as fluid flow, transport issues, physiology, reactor design, statistical analysis, differential equations and numerical methods. In summary, this multi-level experiment demonstrated the integration of various concepts and trains the students seeking a career in medicine or biomedical engineering.

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Biographical Sketches

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