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Experimental Aortic Fluid and Solute Transport Measurement System Independent Study

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Abstract

This paper details the design, development, and preliminary experimental results of a device capable of measuring diffusion through aortic wall samples over time to determine permeability. Initial sketches and 3D CAD designs provided proof of concept. Initial trials showed effective waterproofing of inner and outer drum design in conjunction with the specified O-ring. Overall, **the system was effective in measuring the diffusion across aortic membranes, but improvements to materials as well as experimental procedure would produce more accurate experimental results.**

INTRODUCTION

The objective of the independent study is to design and construct an experimental apparatus capable of measuring the permeability of human ascending aortic wall samples. Studying the diffusion of solutes through the membrane provides insight into the rate and quality of material that the membrane allows to pass through.

The aorta is the largest artery in the human body and is responsible for delivering oxygen-rich blood from the heart from the heart. The ascending aorta extends up from the left ventricle before curves back toward the ground, creating the aortic arch. There are three layers of tissue that make up the aorta; tunica intima, tunica media, and tunica adventicia. The intima (inner layer) consists of smooth muscle tissue, connective tissue and endothelial cells which enable oxygen transport. The media (middle layer) is made up of elastin, smooth muscle tissue and collagen which enables the aorta to dilate to alter blood flow level. The adventitia (outer layer) consists of connective tissue and collagen fibers and serves to anchor the aorta in place. [1]

Knowledge of ascending aortic permeability is important in the study of medicinal solutions to Cardiovascular Disease and, more specifically, aortic aneurysms. An aortic aneurysm is defined as an enlargement (dilation) of the aorta's diameter greater than 1.5 times normal. Aneurysms can occur anywhere along the artery but are referred to as thoracic when occurring in the chest. Often cause by high blood pressure or sudden injury, aneurysms can lead to health complications such as internal bleeding and increased risk of developing blood clots. Insight into aortic permeability will help inform medicine delivery to protect or restore elastic behavior to damaged aortas. [2] [1]

The purpose of the experimental apparatus is to measure fluid and solute diffusion through aortic wall samples. To do this, the wall sample is placed between two separate liquid baths, the donor bath and the sink bath, each with a volume of 6.594 ml. The sink bath contains phosphate-buffered saline (PBS) while the donor is filled with a 1 mg/ml solution of blue dextran in PBS. Assuming the sample is a permeable membrane, solute from the donor bath will diffuse into the sink bath. Aliquots of solution from the sink bath are taken over 4 hours at 30 minute increments. To measure the concentration of dextran in sample solutions, the Spectramax M2E micro plate reader is used. The Spectramax calibration curve is provided in Figure 6. By determining the rate of diffusion of blue

dextran into the sink bath, the diffusion coefficient D and permeability P can be calculated using Fick's Law. Fick's law (Equation 1) states that the rate of diffusion of a substance across unit area (such as a membrane) is proportional to the concentration gradient. [3]

$$J = -D\frac{\partial \phi}{\partial x} \tag{1}$$

Equation 2 shows the formula where dn/dt is the rate of change of dextran molecules over time, A is the surface area of the membrane, and dc/dx is . [4]

$$\frac{dn}{dt} = P * A * \left(\frac{dc}{dx}\right) \tag{2}$$

The system emulates *in vivo* conditions, pressurized to 100 mmHg, similar to average systolic blood pressure. To achieve this, a hydrostatic column pressurizes the system with gravity as it feeds the dextran-PBS mixture to the donor bath. Equations 3-4 show the formula for determining the height required to achieve desired pressure, where P is pressure, ρ is the density of the PBS-dextran solution, h is height and g is gravity.

$$P = \rho * g * h \tag{3}$$

$$h = \frac{P}{\rho * g} \tag{4}$$

METHODS

Table 1 provides all materials and instruments used in the final experimental apparatus. [5] [6]

Item	Company	Serial Number/dim.
Polyurethane O-rings	McMaster-Carr	9558K36
Polyurethane O-rings	McMaster-Carr	9558K37
Quick-turn Female Stopcock	McMaster-Carr	7033T25
Quick-turn Male Stopcock	McMaster-Carr	7033T22
Machine Screw	Home Depot	M4-0.7x55mm
Wingnut	Home Depot	M4-0.7
Vinyl Tubing		0.125 in
Spectramax M2E	Molecular Devices	DEO5933
Blue Dextran	Sigma Life Science	D5751-5G
Pipette	Fisherbrand	DH11479
Phosphate Buffered Saline		100 ml
Microplate		

Table 1   Experimental instruments and materials

Design Process. The initial design process began with research into existing cell membrane permeability measurement devices.[7] After developing a list of constraints and possible design concepts, initial drawings, seen in Figure 1 were produced. Constraints and considerations included separate baths each with cylindrical vents to fit a pipette for aliquots of the solution during experiment. A fluid port to accommodate connection to a hydrostatic pressure column is included as well.
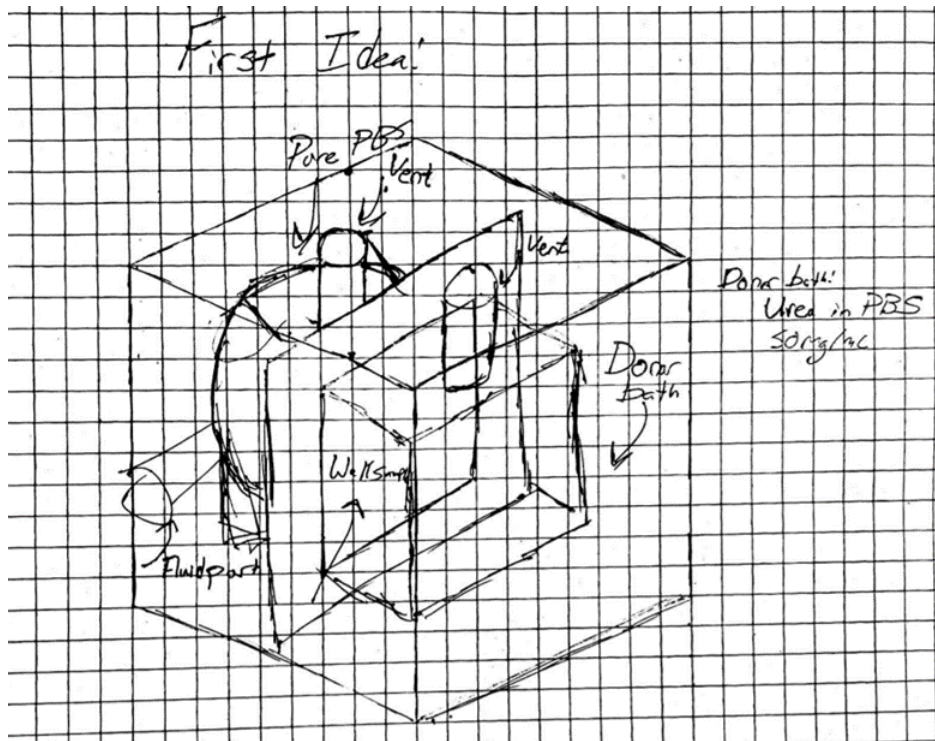
Figure 1   Initial design sketches

Rough mockups of the initial prototype were modeled in Solidworks 3D, shown in Figure 2.
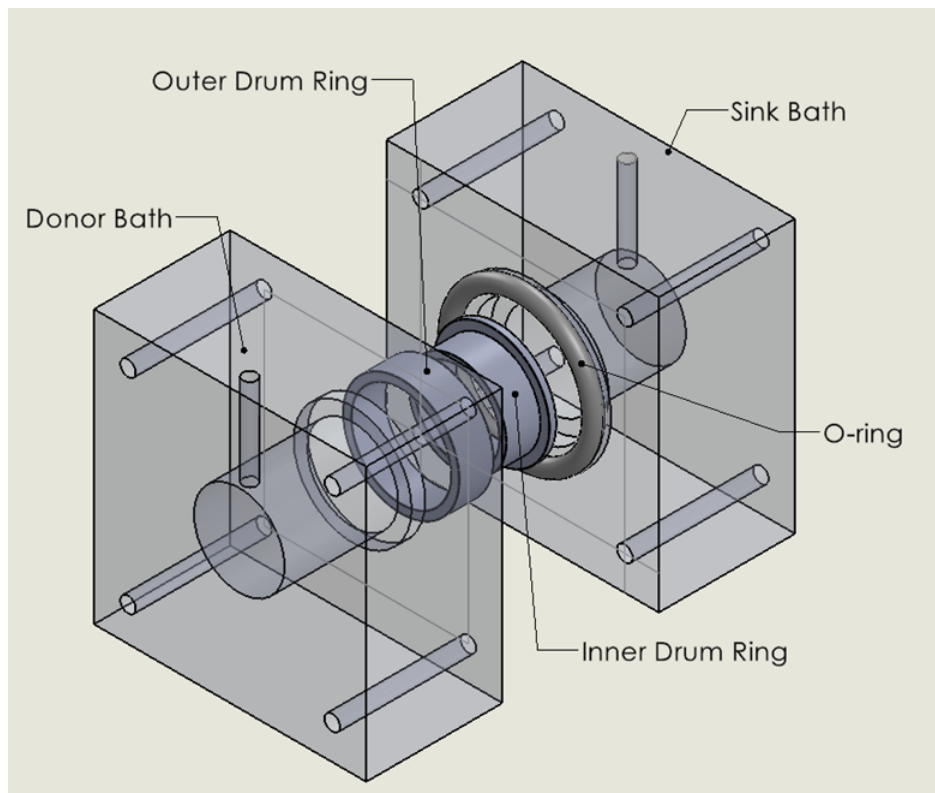

Figure 2   Initial design CAD mockups

At this point, the drum system was developed to securely hold the wall sample between the two baths, with a known surface area exposed to solution on either side. To seal the system and ensure no solution leaks to or from either bath, O-rings are used, positioned around the outer drum ring. Holes are left on each corner to provide a space for an M4 machine screw. With a wingnut, the two sides can be tightened together ensuring the o-ring creates a watertight seal. This initial design was 3D printed using .2 mm thick layers of polylactic acid (PLA). To test the effectiveness of the system, materials with varying thicknesses such as plastic bags and rubber gloves were secured in the system to emulate an aortic wall sample. Solution was pumped into the device without leaks, proving the waterproofing ability of the design. Though functional, this iteration had lots of excess size and material. Additionally, the donor bath must be sealed from the atmosphere in order to maintain pressurized.

The next step in the evolution was a redesign in using Solidworks. For this version, tolerances were manipulated to ensure a tight waterproof seal between parts. Extraneous material was removed, making the apparatus much smaller and sizes were adjusted according to dimensions of the O-rings seen in Table 1. The sink bath was resigned to an open air concept to improve ease of access for stirring and sample taking. A lid was added to prevent spilling or evaporation during an experiment. To account for varying thicknesses of aortic samples, new inner drum rings were printed in a range of sizes to ensure proper fit for any sample, shown in Figure 4. The donor bath was modified as well, with the aliquot opening replaced by a luer lock attachment. The design, shown in Figure 3 was again printed with .2 mm thick layers of PLA.
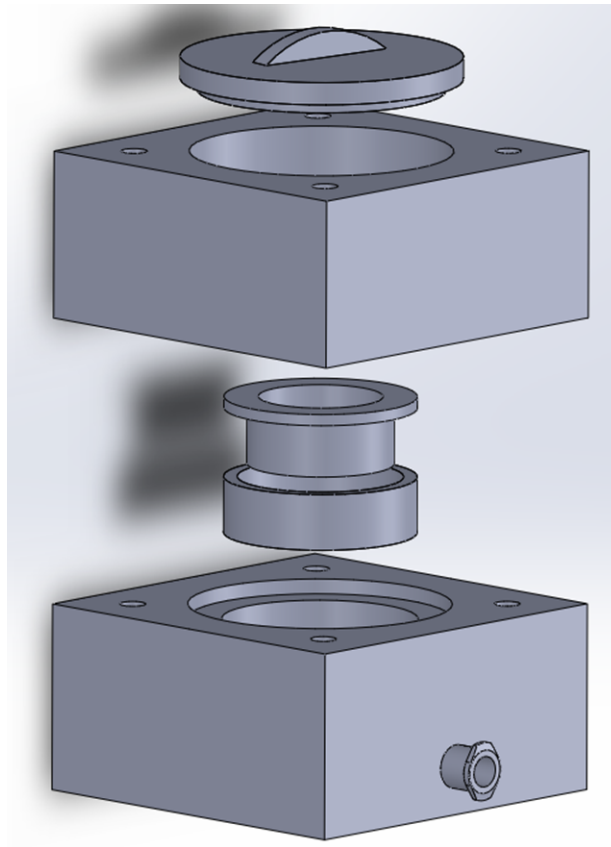
Figure 3   Second design iteration
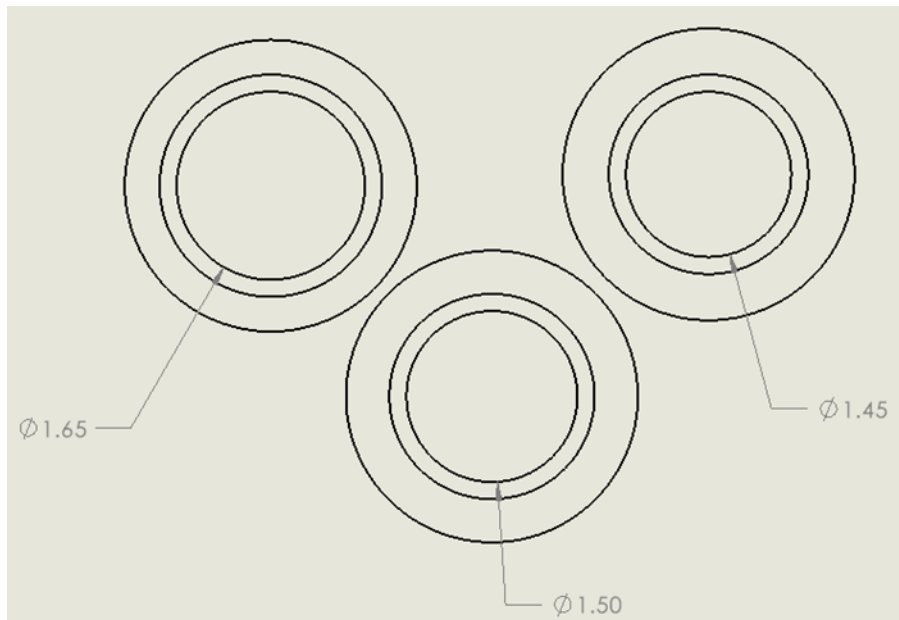

Figure 4   Variety of inner drum rings

This design was fully functional and dimensions fit nicely. The details on the luer lock

connection were too fine for this printer setting, so a final redesign instead included a notch for commercial stopcocks to be glued in, shown in 5. Complete dimensions of the final design can be found in Figure B.1 in the Appendix.
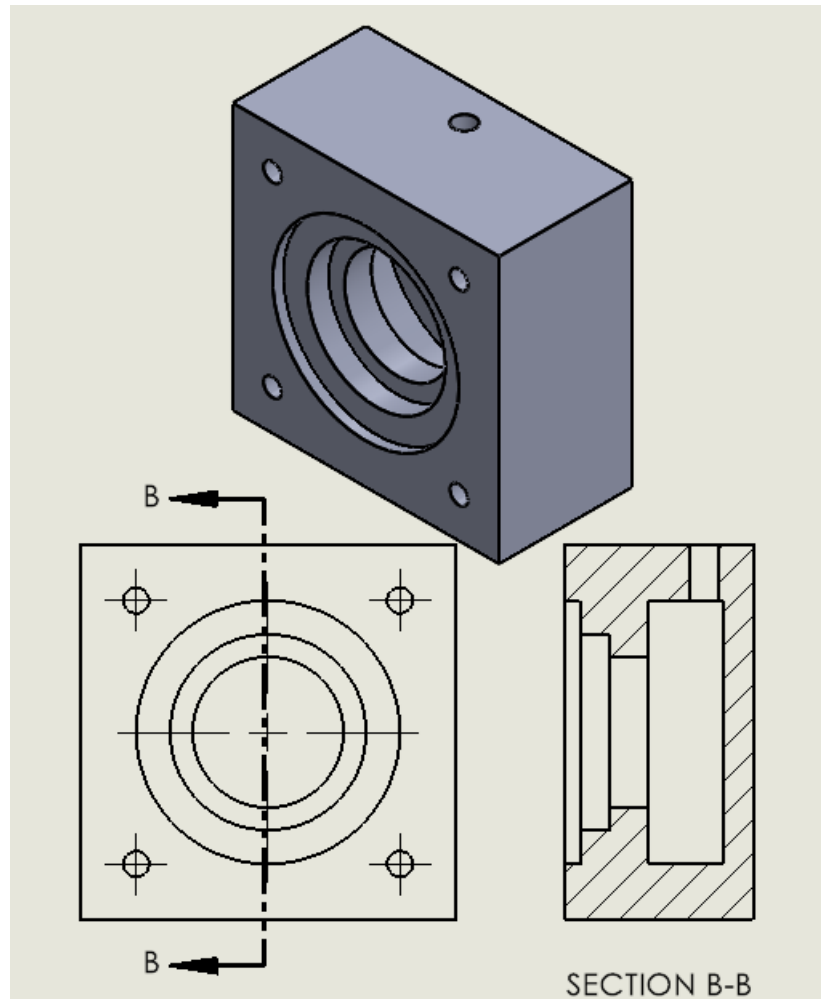

Figure 5   Final donor sink design

This design iteration is fully functional and can be detached, washed and reattached for subsequent trials. Using .1 mm thick layers of Polyethylene Terephthalate - Glycol (PETG), this design was 3D printed and used for experimental trials.

Experimental Procedure. The first step in conducting experimental trials of the apparatus is preparing the necessary materials. For these trials, ascending aorta are dissected from pig hearts and used in place of human samples. The adventitia is removed from the artery which is cut into circular samples 3 cm in diameter and stored in PBS. Blue dextran is massed to 100mg and added

to 100ml PBS. To calibrate the Spectramax, 50μl of blue dextran is added to the first two wells of a microplate using a pipette. The first well represents the original concentration of dextran. 50μl of PBS is added into the second well and stirred until solutions are fully combined. From the mixed second well, 50μl is removed and placed into the third well. Steps of adding PBS, mixing and moving to the next well are repeated until wells 1-7 contain 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625% dextran concentration respectively. The eighth well is filled with 50μl of PBS. The microplate is measured using the Spectramax and results are plotting and fit with a trend line to create a calibration curve, as seen in Figure 6. Raw data can be found in Figure A.1 in the Appendix.
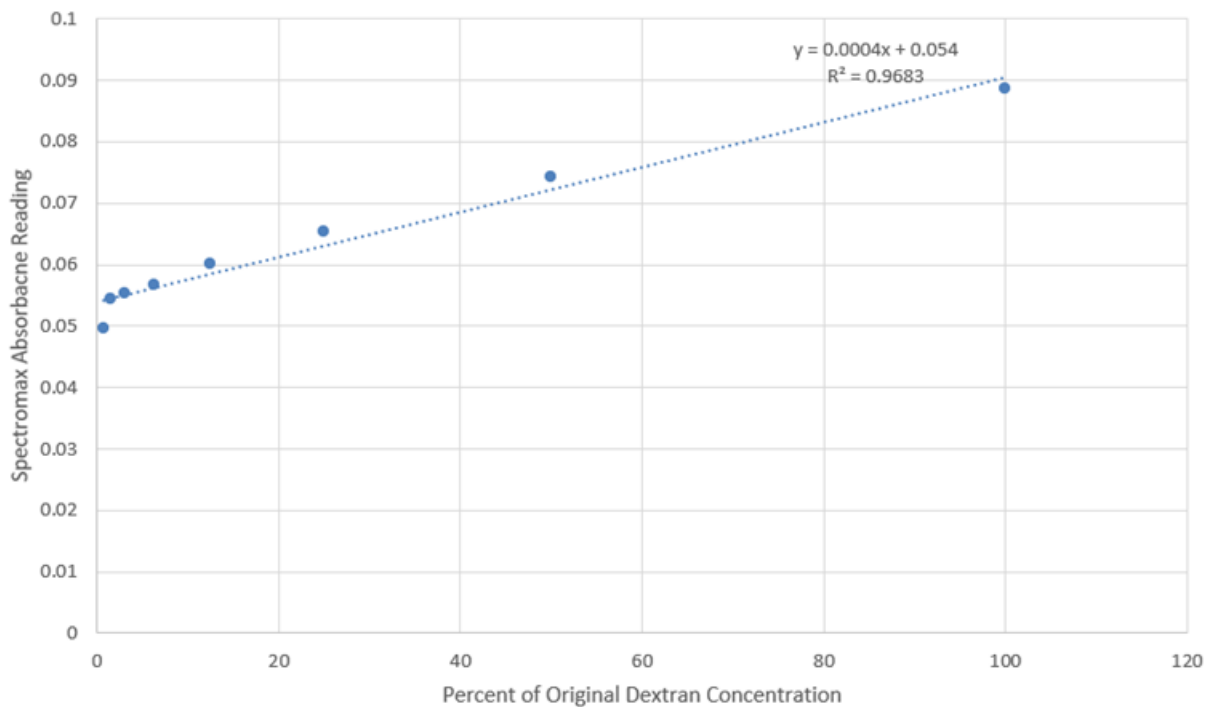

Figure 6   Original Spectramax calibration curve

Including all data points provides a trend line with an R-Squared value of 0.9683. By only including the more linear region of the calibration data, a more accurate trend line with R-Squared value of .9895 is achieved, shown in Figure 7.
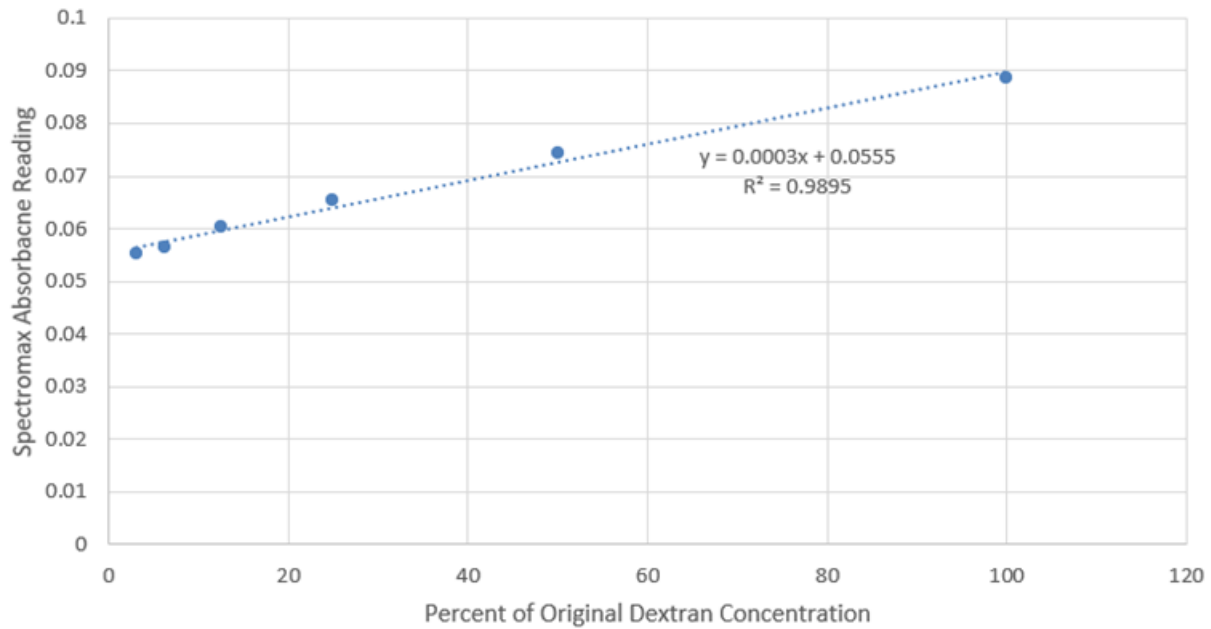
Figure 7   Second Spectramax calibration curve

Next, the experimental apparatus must be set up. With the aortic sample prepared and the sink bath hooked up to the hydrostatic pressure column, the stopcock should be opened to fill the donor sink with the dextran solution until it is level with the smallest of the concentric extrusions. The circular aortic sample should be placed concentrically on top of the inner drum ring. The outer drum ring can then be slid over the sample. Note that an appropriate sized inner drum ring will provide some resistance to sliding on the outer ring, but should not stretch the sample significantly. With the sample clasped into the drum, it can be placed face down into the inner most ring such that one side of the sample begins to touch the dextran solution. The sink bath can then be placed on top and tightened using the wingnuts. Once tight, PBS is added to the fill line, the pressure column stopcock is opened and the experiment timer can be started. The first aliquot is taken and placed into the microplate, then taken again every 30 minutes for 4 hours. These microplates are then measure using the Spectramax to provide diffusion figures.

RESULTS & CONCLUSION

Results from 2 experimental trials using the PETG apparatus are shown in Figure 8. In these experiments, two aliquots are taken from the sink bath at every 30 minute increment.
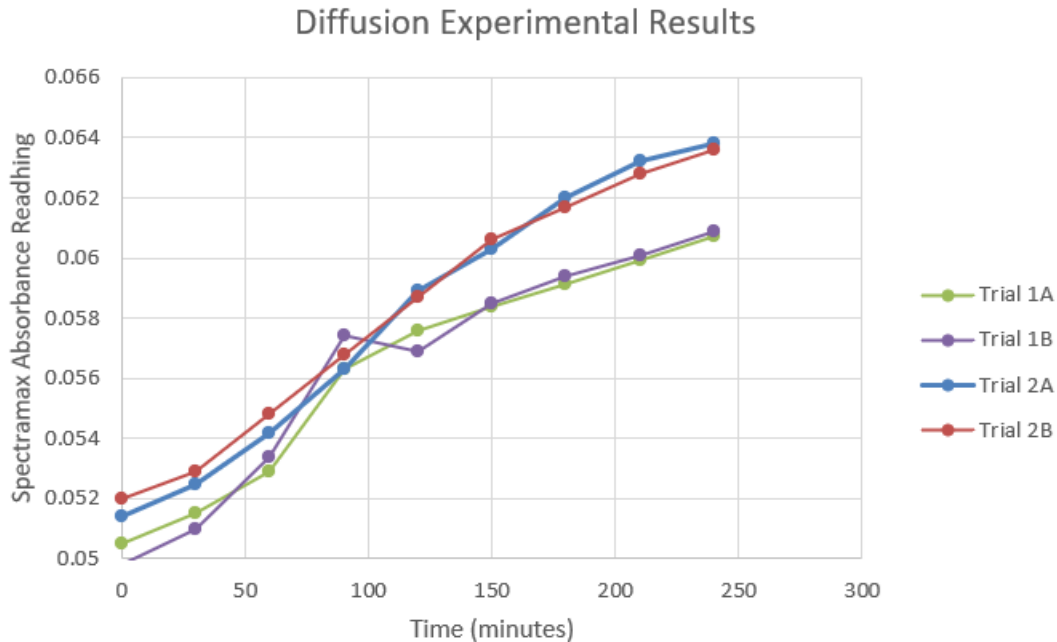

Figure 8   Experimental Diffusion Results

For diffusion through a permeable membrane it is expected that solute will diffuse from areas of high concentration to areas of low concentration until the system reaches equilibrium. In this case, it is expected that dextran would diffuse from the sink bath to the donor bath until the to are in equilibrium. 100 ml of 1mg/ml dextran solution is added on the donor bath side, and 20 ml of PBS sit on the donor side. For equilibrium conditions, we could then expect a sink bath concentration of 83%. Our the calibration curve, this would correlate to an absorbance reading of approximately 0.0830. After 4 hours, the highest experimental absorption reading is only .0638, approximately 25% dextran concentration. When compared to published figures for diffusion of other materials through aortic walls and other arterial tissue, it becomes clear that the diffusion in this experiment is occurring at a much slower rate than expected.[8] [9]. Each trial yielded a slightly s-shaped curve meaning diffusion was initially slow, sped up in the middle of the trial, and began to slow again towards the end.

Assumptions made during the experiment include constant thickness throughout each

membrane. The average human aortic wall is 2.67±0.27 mm.[10] This range of plus/minus 10% can affect diffusion rates significantly. The assumption is also made that the adventitia is fully and evenly removed from the tissue sample. This layer of connective tissue and collagen fibers would disrupt any diffusion if not fully removed, which may have been the cause of slow diffusion in the experiment. It is also assumed that the sink bath is well mixed and aliquots are accurately representative of solution as a whole. Inadequately mixing the solution would lead to discrepancies in reading between A and B samples of a trial. Additionally, we are assuming that the apparatus itself is not permeable. This is likely the largest source of error in these experimental trials. After 2 trials, the donor sink of the previously grey PETG was dyed blue by the dextran. It is likely that during the experiment solute seeped into the porous PETG material and was no longer able to diffuse. To combat this, a few steps can be taken. Allowing the trial to run longer to ensure the system is at steady state may show more developed diffusion. Machining the same system out of acrylic could also prevent the dextran from attaching to the sink. Next steps would include conducting experiments with the newly produced acrylic apparatus, as well as using actual human aortic samples in experiments.

References

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Appendix

A Raw Data

	1	2	3	4	5	6	7	8	9	10	11	12
25.4	0.0974	0.097	0.074	0.0738	0.0684	0.068	0.0636	0.0531	0.0576	0.0581	0.0576	0.0576
	0.0979	0.0761	0.0669	0.0638	0.059	0.055	0.0576	0.0503	0.0576	0.0573	0.0583	0.0578
Calibration	0.0887	0.0743	0.0654	0.0602	0.0566	0.0553	0.0545	0.0497	0.0581	0.0571	0.0588	0.0575
Trial 1 A	0.0505	0.0515	0.0529	0.0563	0.0576	0.0584	0.0591	0.0599	0.0607	0.057	0.0643	0.0577
Trial 1 B	0.0498	0.051	0.0534	0.0574	0.0569	0.0585	0.0594	0.0601	0.0609	0.0571	0.0573	0.0576
	0.0575	0.0575	0.0573	0.0575	0.0599	0.0575	0.0576	0.0576	0.0573	0.0572	0.0573	0.0578
Trial 2 A	0.0514	0.0525	0.0542	0.0563	0.0589	0.0603	0.062	0.0632	0.0638	0.0574	0.0576	0.0579
Trial 2 B	0.052	0.0529	0.0548	0.0568	0.0587	0.0606	0.0617	0.0628	0.0636	0.0581	0.0586	0.0579

Table A.1   Raw absorbance data

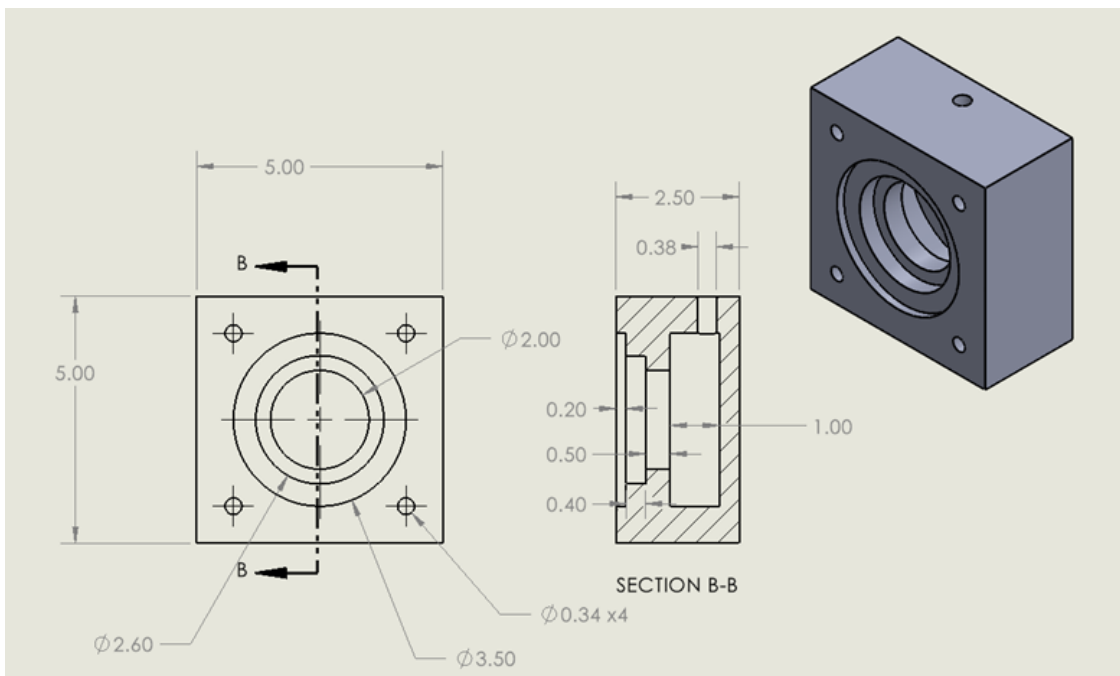## B Final Dimensions

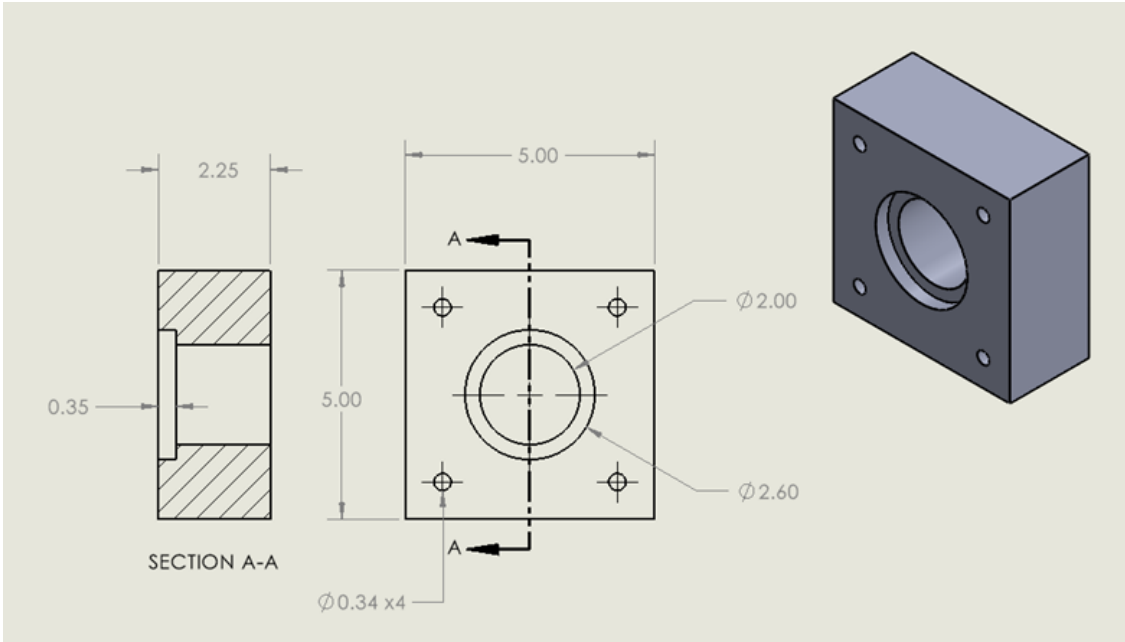**Figure B.1   Final donor bath dimensions**

Figure B.2   Final sink bath dimensions

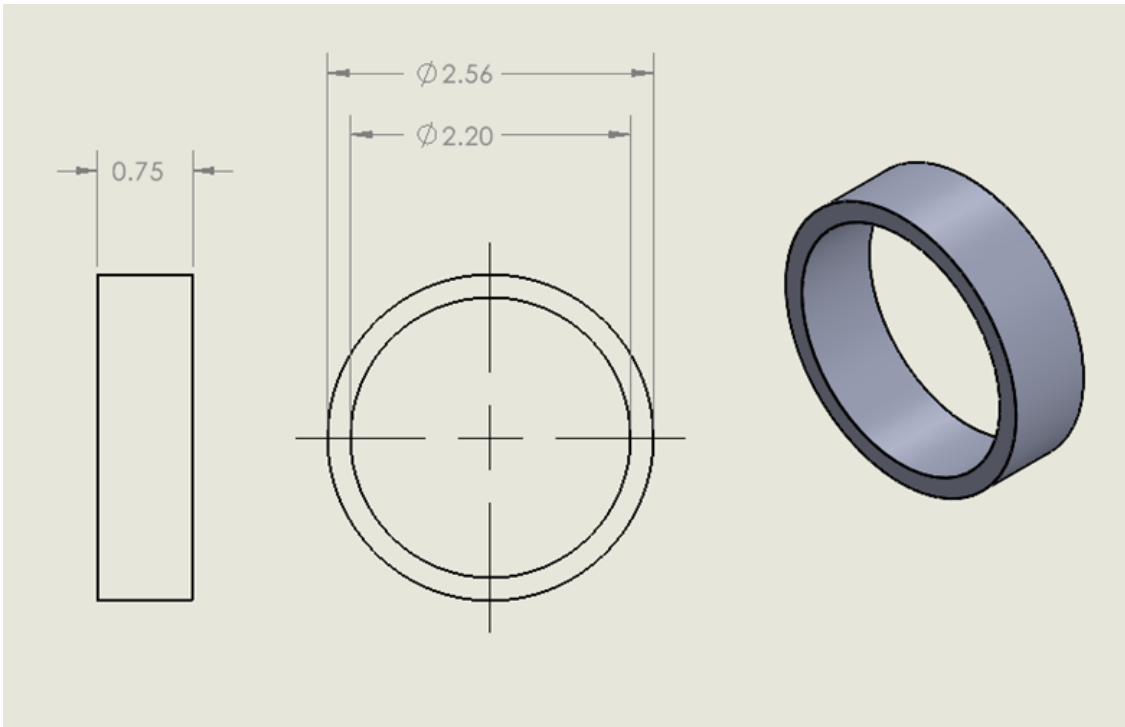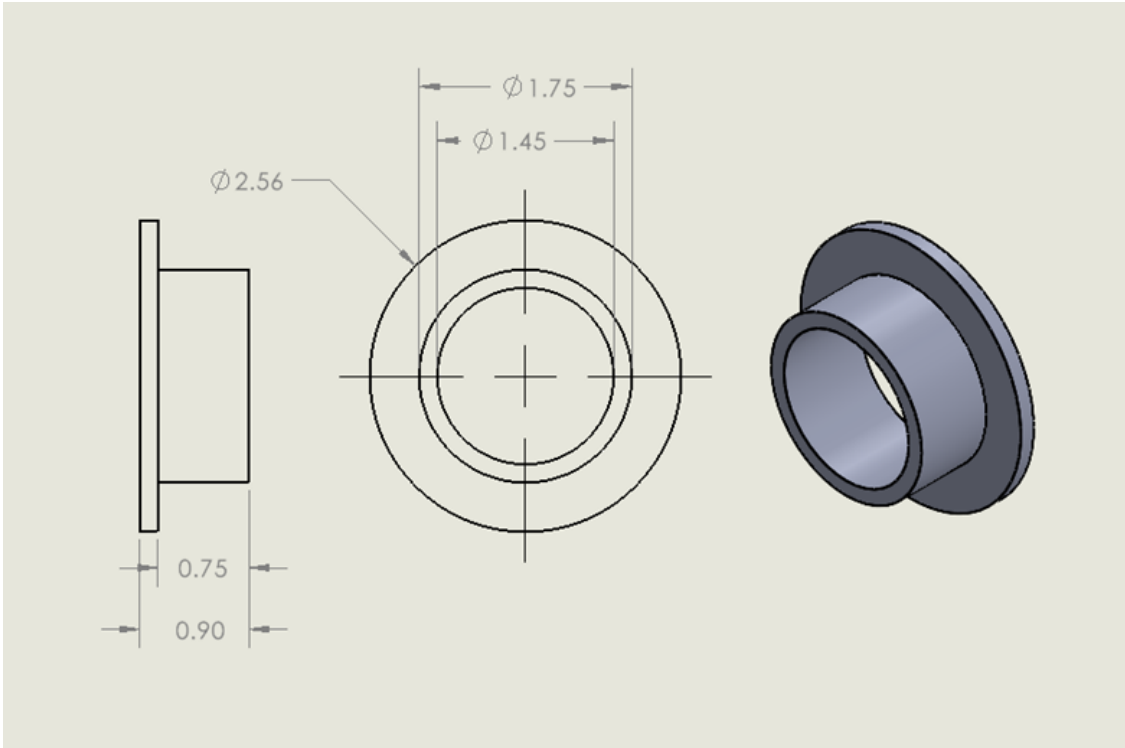**Figure B.3   Final outer drum ring dimensions**

Figure B.4   Final inner drum ring dimensions