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Efforts in increasing Microdialysis recovery rates by utilizing bidirectional flow capabilites

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Efforts in Increasing Microdialysis Recovery Rates

by Utilizing Bidirectional Flow Capabilities

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Table of Contents:

I-Abstract

Microdialysis sampling involves the collection of biological fluids from tissues or fluid-filled cavities *in vivo* via diffusion through a semipermeable membrane probe. In order to increase the recovery of fluids for analysis from this process, a new regime was attempted that would allow perfusion fluids to make multiple passes through the probe in order to collect more analyte with each additional pass. This was dubbed the Bidirectional Flow Technique. Dextran-70 solution was used as the perfusion fluid while 100μM Methyl Orange solution was used as the analyte. The experiments were performed *in vitro* using a fully automated microdialysis ePump capable of performing the bidirectional fluid pushing and pulling. Samples were collected on a range of 1-11 passes of perfusion fluid through the membrane. The recovery of analyte increased with each addition of two passes at rates averaging to a linear progression of +5.45% recovery per added pass. Upon further experimentation, it was noted that the amount of analyte recovered from the backwards passes was far lower than that of the forward passes, a phenomenon thought to be an effect observed due to the construction of the semipermeable probe having not been built with this type of passing regime in mind.

II-Introduction

A. Microdialysis

Introduced in the 1970s[1], microdialysis sampling has become a common method of analyte collection used in clinical settings. Microdialysis sampling is a diffusion based technique that involves the collection of biological molecules for analysis through the implantation of small semipermeable probes in living bodily tissues[2] or fluid cavities[3] that contain the targeted molecules to be analyzed. As illustrated below, perfusion fluid travels through the inlet tubing into the semipermeable membrane of the probe where it passes out of the tip and into the extracellular matrix. With a lack of analyte inside the pump, this creates a concentration gradient by which analyte will passively diffuse into the membrane and then travel through the outlet tubing into a collection vial for analysis[4]. The resulting dialysate fluid, a combination of perfusion fluid and analyte, can be collected at various times and have its content analyzed.

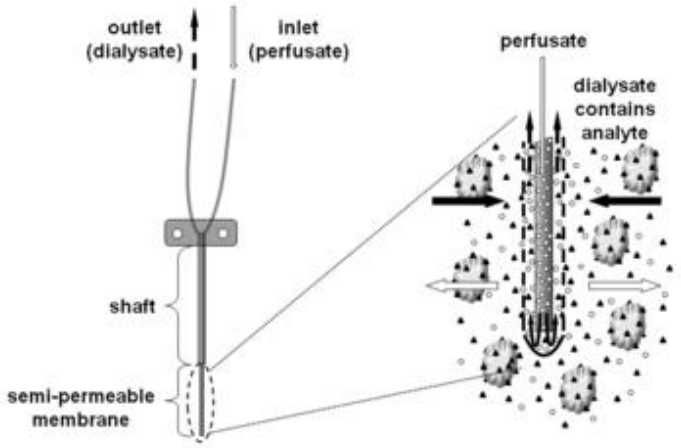

Figure 1: Diagram showing the parts of a microdialysis probe, diffusion pattern, and the direction of flow

Microdialysis probes are normally designed for collection of low molecular weight substances on the order of 5-30kD, but certain designs allow for up to 1MD in order to collect larger substances of interest[5]. Microdialysis techniques have been widely employed to serve various areas of biological sampling, not only in test animals, but also in human subjects as well[6]. In the realm of neuroscience, it is used to study neurotransmitter release[7] and help combat degenerative diseases such as Parkinson's and Alzhiemers by providing a mechanism to view changes of molecules located in the brain fluid after the addition of experimental treatments[8]. Various uses are also being seen outside of the brain in areas such as sampling and analysis of spinal fluid, adipose tissue, muscle fibers, and liver tissue[1].

B. Recovery and Theoretical Background

Recovery, based on the equation below, refers to the concentration of analyte in the dialysate fluid in relation to that in the fluid surrounding the probe.

$$Relative\ Recovery = \frac{C_{outlet} - C_{inlet}}{C_{solution} - C_{inlet}}$$

The C_{outlet} values is rarely equal to the $C_{solution}$ value due to the constant flow of perfusion fluid through the probe hindering equilibrium of the fluids inside and outside the probe[4]. Recovery is used as a gauge for how efficiently the probe is working. The higher the recovery, the more analyte is diffusing into the probe giving a higher concentration for analysis. Experiments are normally conducted at a flow rate of 0.1-5μL/min in order to attain an appreciable recovery. These flow rates are necessary in order to compensate for the residence time of fluids in and around the probe, the time it takes for analytes to move from outside the probe to the inside and vice versa for perfusion fluids. Also, resistance to the process is introduced from the inlet fluid, outlet fluid, and the solution containing analyte, even more hindering the speed of the process.

In order to increase the accuracy of the process and attain better samples for analysis, efforts have been made to increase the recovery of microdialysis probes. Some solutions have been found to be effective including lowering the flow rate and increasing the membrane pore size of the probe[4]. While these are effective solutions, they do present certain issues. With a lower flow rate, now experiments take even longer, so if time is an issue, this will not be helpful. As for increasing pore size, an advantage to microdialysis is that the diffusion was based on molecular weight. With a larger pore size, some extra molecules may be picked up and deposited in the dialysate that are not wanted.

Therefore, it was the goal of my experiments to find a way to increase the recovery of microdialysis sampling experminets without compromising time or altering the probe in use. The idea presented to me was that of multiple passes of perfusate fluid through the probe. A sample of perfusion fluid would flow from the pump, thorugh the probe, and into the collection vial (this would be known as the forward flow). From here, fluid in the collection vial would then be pulled back and passed through the probe again in order to collect more analyte (this would be known as the backward flow). This technique was dubbed the bidirectional flow technique

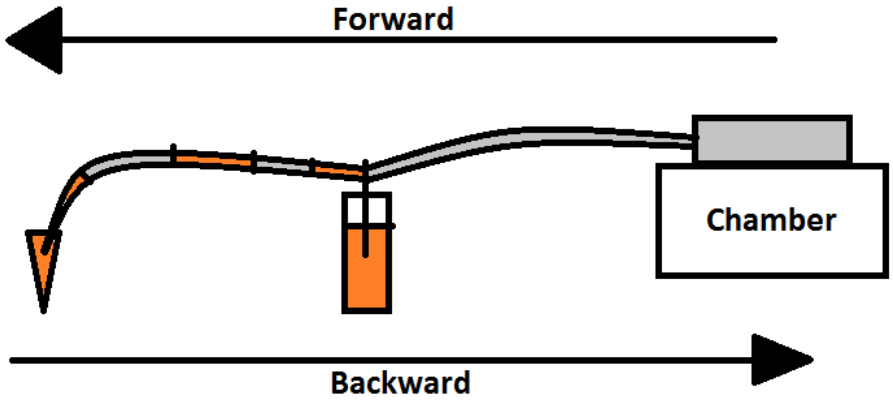

Figure 2: Diagram showing the proposed pathway for flow of perfusion fluid in both directions through the microdialysis probe.

C. UV-Vis Analysis

UV-Vis (Ultraviolet-Visible) analysis refers to absorbance spectroscopy taking place in the regions of ultraviolet and visible light. Light is passed through the analyte at a known intensity and some of this light is absorbed by the analyte. The amount of light absorbed and the reduction of intensity measured gives an absorbance value[9]. The measurements taken of the sample measure the difference from ground to excited states of the charged electrons in the analyte[10]. For our purposes, a Nanodrop 2000c spectrophotometer was used for the analysis.

D. SFC Fluidics ePump

In order to assess the possiblility of the bidirectional flow technique, it was necessary to find a tool that allowed for the possibility of the regime to be implemented. The ePump allows for continuous flow over a large range of flow rates and can be fully programmed to be run with any computer equipped with the user software. Apart from the mechanical additions, the pump behaves very similarly to a normal syringe pump[11]. By taking advantage of the automatic refill command, the ePump can be used to pull fluid from the outlet tubing, into the membrane, and end in the inlet tubing from the chamber, simulating a backwards flow. Full automation of the pump also allows for a continuous flow of fluid without the need to stop and reset the fluid between each passage through the probe.

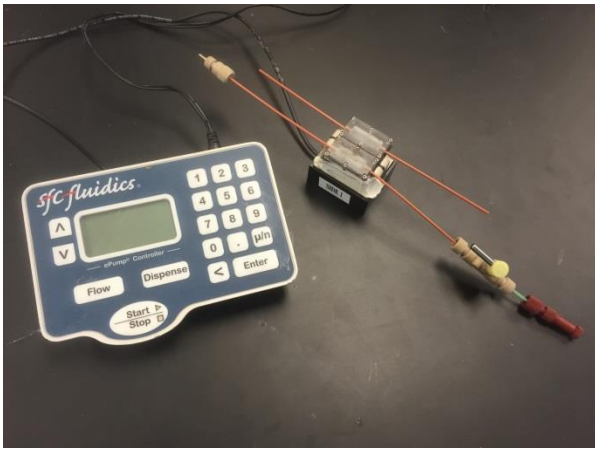

Figure 3: Picture showing the fully assembled ePump and chamber.

III-Materials and Methods

A. Chemicals

Methyl orange powder, dextran (from Leuconostoc spp.), 10mM phosphate buffered saline (PBS) of pH 7.4 was used and its ingredients were purchased from Sigma Aldrich (St. Louis, MO). HPLC grade water was purchased from Fischer Scientific (Fair Lawn, New Jersey).

B. Equipment

The ePump used was from SFC Fluidics (Fayetteville, AR). The BAS syringe and syringe controller were purchased from BASi (West Lafayette, IN). The Nanodrop 2000c was purchased from Thermo Scientific (Waltham, MA). The CMA 20, membrane length 4mm, microdialysis probe with polyethersulfone (PES) membrane and 100kDa MWCO was obtained from CMA/Microdialysis AB (Holliston, MA).

C. Preparation of Solution

Sodium chloride, potassium chloride, sodium phosphate, and potassium phosphate were mixed to make 10mM PBS solution. Methyl orange reagent was mixed with the 10mM PBS solution to make 100µM methyl orange solution. Dextran-70 was mixed with HPLC grade water to make a 4% dextran solution.

D. Main Experimental Procedure

Dextran solution, the perfusion fluid, was loaded into the empty chamber of the ePump, using either a special 10 mL syringe that screwed into the ePump or with a normal BAS syringe outfitted with connecter pieces that screwed into the

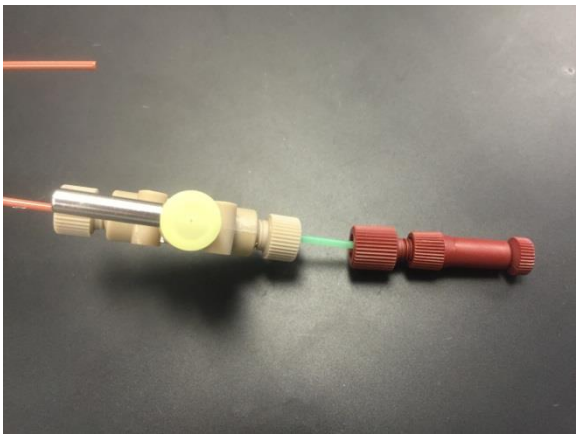

Figure 4: Picture showing the specialized connection apparatus used to add perfusion fluid into the chamber.

chamber, until the chamber was full. The CMA 20 probe was inserted into a 1.5 mL vial filled with 100 μM methyl orange in 10mM PBS solution and connected via inlet tubing to the ePump. The ePump was connected to a laptop equipped with a program used to run the pump remotely. The dextran solution was passed from the fluid chamber, through inlet tubing to the probe, and emptied into a collection vial as shown in the figure below [1]. All experiments were performed at a flow rate of 5 μL/min. Using the Nanodrop 2000c, the dialysate was analyzed via UV spectrometry to determine the amount of methyl orange recovered along with the dextran solution. A calibration curve was made to determine the concentration of the samples with 10mM PBS solution used as a blank. The Dextran-70 solution was used as the blank during actual experimentation since this was used as the perfusion fluid. The ePump was programed to follow the following pattern that corresponds to 2 passes, and to repeat for the specified number of passes, refer back to Figure 2 for a visual representation.

1. Flow forward for 3 minutes
2. Stall for 1 minute
3. Flow backward for 2 minutes

E. Additional Experimental Procedures

An experiment was performed in order to assess the amount of methyl orange recovered on the backward flow using both the ePump as well as a normal BASi syringe. This was completed in the same fashion as the normal passing experiments, with minor alterations. Since fluid must be passed forward in order to pull backward, the probe was placed into a vial contained the same fluid as the chamber, dextran-70 solution. This essentially acted as a "blank" for the forward pass, with no methyl orange being picked up. Then before

the backward pass started, the probe was moved into a vial of methyl orange, so that some could be collected as the backward pass was performed. The ePump was programed with the same time allotted as in the normal experiment and both the Pump passing and the BASi syringe passing were completed at a flow rate of 5 uL/min. This was continued for as many passes as needed. The Nanodrop 2000c was used for this UV analysis as well.

IV-Results

The following graph shows a calibration curve used for comparison when collecting samples of unknown concentration. A new curve was created before each experiment in order to make sure that both the pump and Nanodrop were performing correctly.

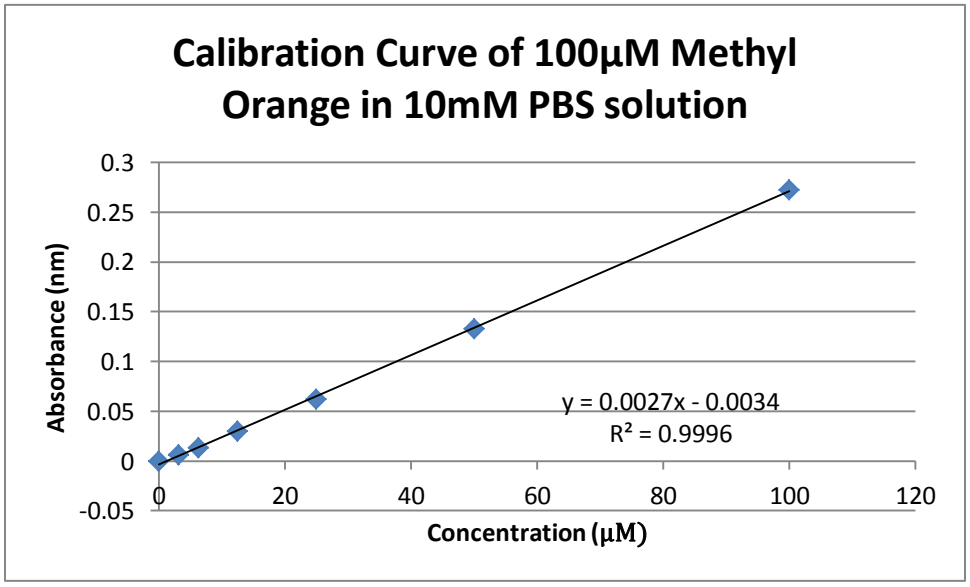

Figure 5: Calibration Curve showing absorbance of methyl orange solution diluted with PBS vs concentration of methyl orange obtained from UV-Vis analysis.

Plausibility/Procedure in Practice

In order to assess the validity of the hypothesis, that making multiple perfusion fluid passes through the probe would collect more analyte, the experiments featured in Figures 6 and 7 were performed. In these experiments, fluid was passed through the probe up to 5 times, with both forward flowing and backward flowing each counting as individual passes. The attained data shows an increase in recovery for both trials of the experiment, prompting the notion that the bidirectional passing regime does produce an increase in analyte recovery. All passes were performed at a flow rate of 5μL/min in order to be able to attain the largest number of data points.

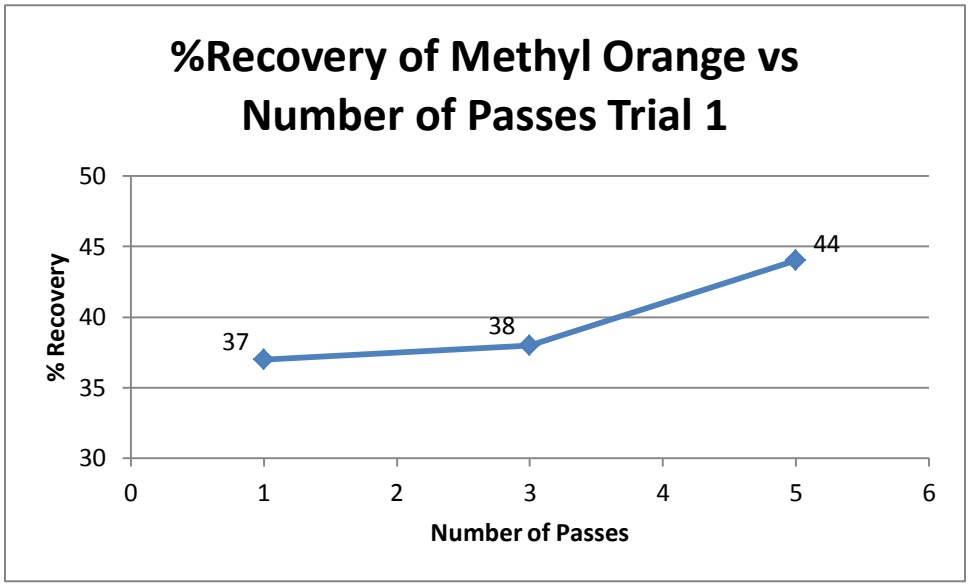

Figure 6: % Recovery vs Number of Passes for Methyl Orange, first trial for bidirectional passing.

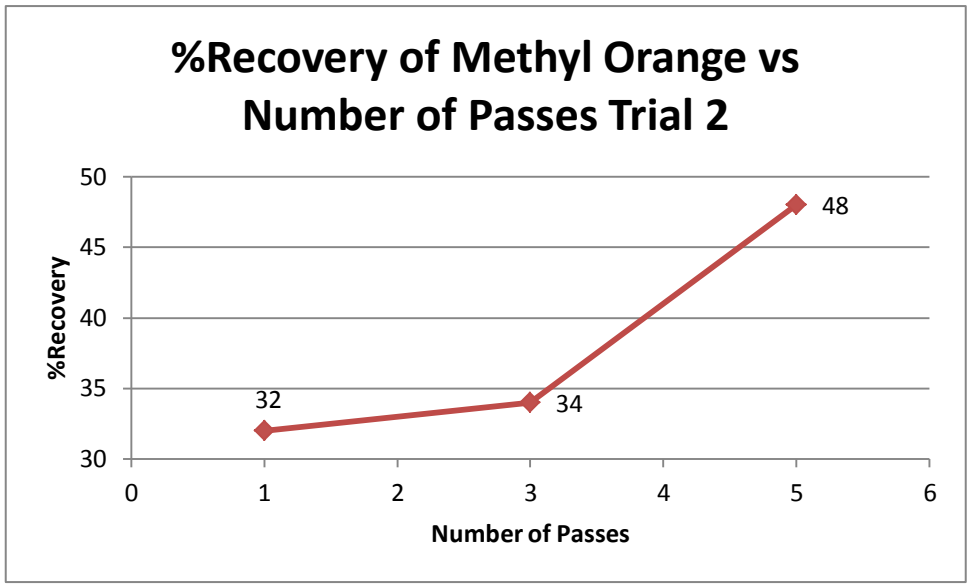

Figure 7: %Recovery vs Number of Passes for Methyl Orange, second trial for bidirectional passing.

The cause of the low recovery gain between 1 and 3 passes is not known for sure, but it is hypothesized to result from the probe being used. After moving on to new experiments and switching to a new CMA 20, more linear gains were noticed, implicating the original probe as the source of error. Figures 6 and 7 show 5 passes giving close to 50% recovery, so it was thought that adding another 5 passes, at the same flow rate of 5µL/min, would result in close to 100% recovery. 11 passes was settled on as the new pass number since an odd number had to be chosen for the purposes of collection dialysate.

Increasing Number of Passes

Figures 8 and 9 show the data obtained from passing 1-11 times through the probe. An increase in analyte concentration was attained through 11 passes ending in approximately 80% on both trials. All passing experiments were performed at the same flow rate of 5µL/min for the sake of continuity. No experiments were performed at lower flow rates; however it is hypothesized that these recoveries would be higher than those shown here. In normal microdialysis sampling, with no bidirectional regime implemented, lowering the flow rate increases the recovery of analyte, so

it is safe to assume the same trend would be observed here. For the first trial, the non-linear trend continued between passes 1-3 and 7-9. Since the overall recovery increased with each pass, this is thought to be an issue with the flushing of the pump. On the second trial, a much more linear trend is observed, supporting the thought that the non-linear areas of previous trials were pump errors.

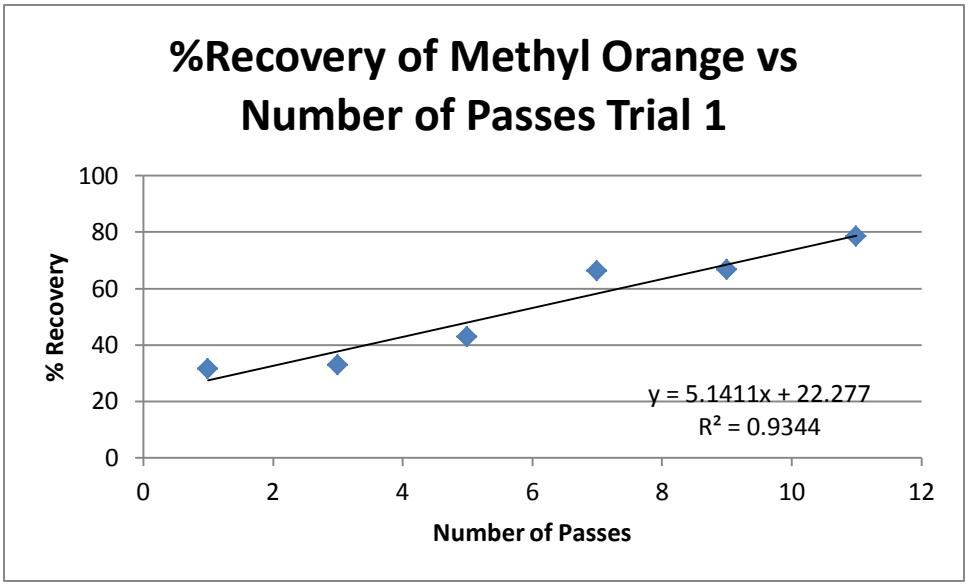

Figure 8: %Recovery vs Number of Passes 1-11 for Methyl Orange on first trial of bidirectional flow.

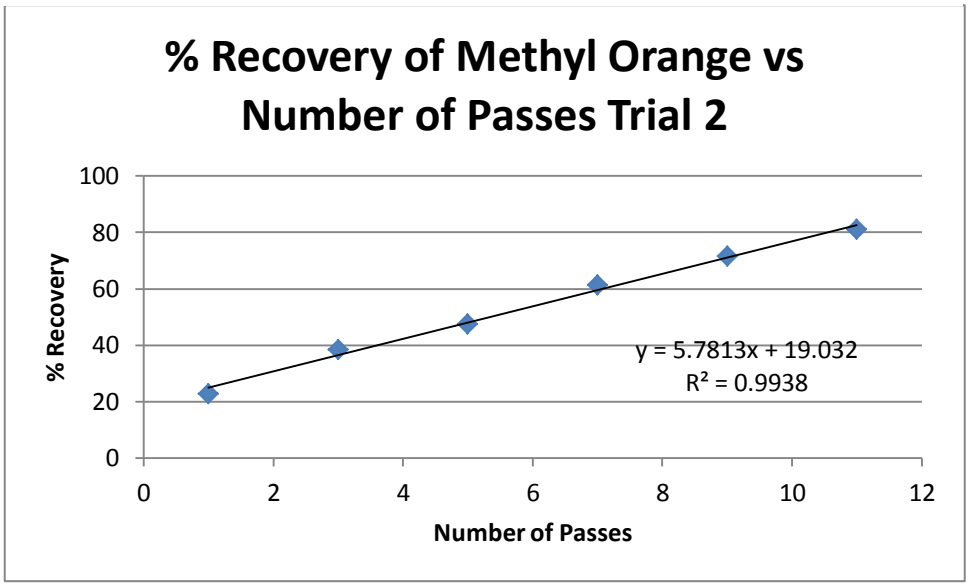

Figure 9: %Recovery vs Number of Passes 1-11 for Methyl Orange on second trial of bidirectional flow.

Backwards Passing

Figure 10 shows the results of the backwards passing experimentation. By comparing the values in Figure 10, showing backwards only recovery, with those of Figures 6-9, showing forward and backwards working together, the difference in recoveries ranges from 20-30% recovery. This means that the backwards flow is only contributing 20-28% of the analyte collected, making it far less effective than the forward passing. It is believed that this is the case because of the design of the microdialysis probe. The probes are designed to be very efficient passing fluid from the inlet to outlet tubing but not necessarily in the reverse direction. An alteration in the design of the probe would be the best way to increase the efficiency of the backwards flow.

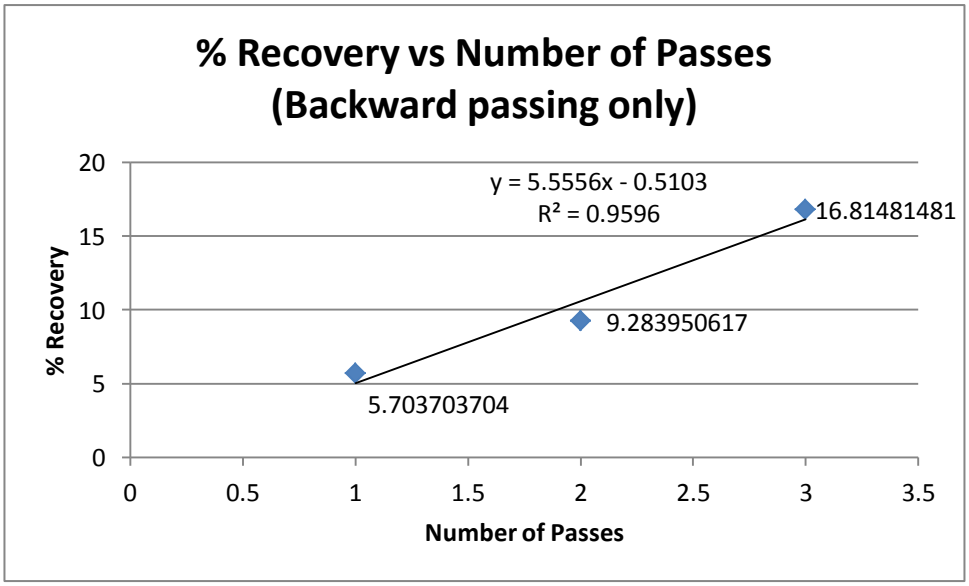

Figure 10: Results of backwards passing experimentation. Illustrates how forward passing is currently more efficient.

Figure 11 shows the results of the same experiment performed using a manual BASi syringe pump. The experiment was performed in order to have a comparison for the results of Figure 10 and see if these results were caused by the ePump.

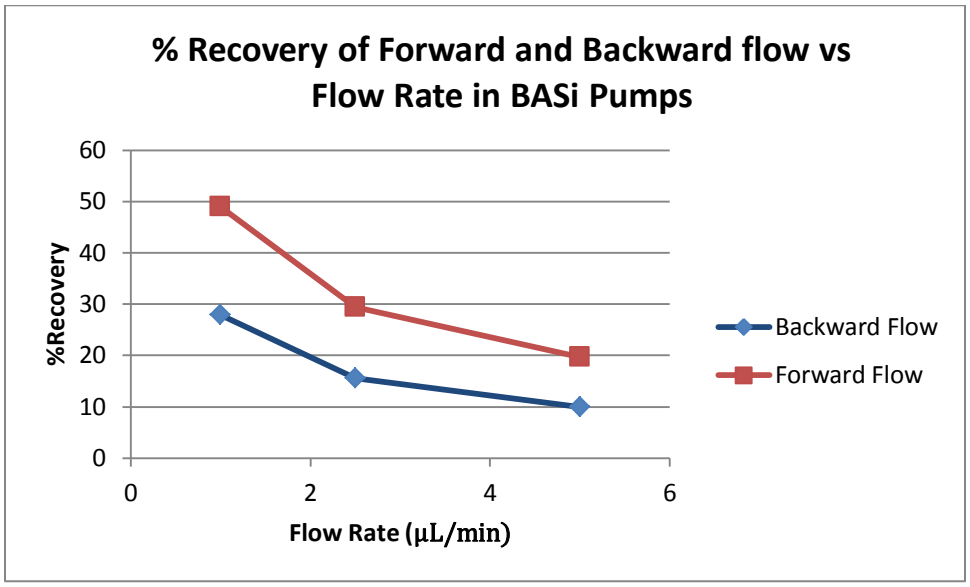

Figure 11: %Recovery of both Forward and Backward flows vs flow rate using a BASi manual syringe pump.

The results show that the backward flow is not as effective as the forward flow in the manual pump, corroborating the results obtained from the experiment performed with the ePump. This experiment also observed that at lower flow rates, the probe was able to give a higher recovery.

V-Conclusions

The bidirectional flow technique was shown to be a viable flow regime for increasing the amount of analyte recovered from *in vitro* samples. At a flow rate of 5µL/min, 80% recovery of analyte was consistently recorded at 11 passes through the probe with higher recoveries predicted if a lower flow rate was used. Upon further investigation, the backwards flow was found to collect far less analyte than the forward flow. This is thought to be due to the design of the CMA 20 probe. Inside the probe, two tubes extend into the membrane, one from the inlet and one from the outlet tubing. The outlet tube is far shorter than the inlet tube so that it is easier to uptake dialysate into the outlet. If the outlet tube was extended and the inlet tube shortened, so they were the same length, the recovery of analyte from backwards flow should increase to the level of the forward flow. Future experimentation should test these experiments against other flow rates in order to assess whether these lower rates give increased recoveries using the same number of passes. Also, other probes should be tested to see if the same discrepancy between the forward and backwards flow exists.

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