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Percent Recovery of Various Analytes Using a Wick Method

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Percent Recovery of Various Analytes Using a Wick Method

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Table of Contents: Page Number(s)

Abstract:

Finding alternate methods to measure and isolate interstitial fluid in vivo has become a consequence of the limited recovery of analytes when using a probe.[1] These probes consist of an inlet constantly bringing perfusate into a semi-permeable membrane where the perfusate is replace with dialysate containing numerous analytes. The dialysate leaves the semi-permeable membrane via an outlet. A wick technique has been shown to be useful in analytical chemistry because it can be used to effectively isolate interstitial fluid and accurately measure the concentrations of various analytes. For long-term applications, wicks can be used to measure the concentrations of cytokines and other small proteins in vivo.

This research focuses on obtaining the percent recovery of methyl orange and FITC-4000 using a wick technique in vitro. The wick technique used in these experiments consisted of extracting the fluid from the wick using a centrifuge method and then analyzing the fluid using UV-Vis Spectrophotometry. It was determined that the percent recovery for methyl orange using the wick technique was 88.3% ± 1.4% (n=9). Within methyl orange, it was experimentally found that the percent recovery of methyl orange for 1 cm unwound wicks, 3 cm unwound wicks and 3 cm wound wicks was 90.1% $\pm$ 1.7% (n=3), 90.4% $\pm$ 3.0% (n=3), and 79.8% $\pm$ 1.7% (n=3) respectively. The percent recovery for the FITC-4000 using the wick technique was determined to be 84.5% $\pm$ 1.5% (n=9). The results of this work suggest that the wick technique used in these experiments can be used to efficiently recover various small analytes.

Introduction:

Microdialysis is a sampling technique that continuously measures analytes in interstitial fluid. Accurately measuring the concentration of cytokines, small proteins, and other analytes using a probe in vivo can be a challenging endeavor when using microdialysis. Measuring the concentrations of analytes with a probe in vivo is difficult because the probe's recovery is limited by the flow rate, and it can be challenging to have the ideal flow rate in vivo. It is also absolutely critical to isolate fluid that is representative of the interstitial fluid in many different disciplines and a variety of methods have been created for fluid sampling. One promising method for fluid sampling, however, is the wick method.

Various wick techniques have been shown to accurately measure the concentration of a variety of different analytes in vivo. Aukland and Fadnes presented the original wick method. [2] In the original wick protocol proposed by Aukland and Fadnes, the wicks were soaked in saline and implanted into rat subcutis in vivo. These experiments by Aukland and Fadness were the first experiments that attempted to measure the concentration of analytes in vivo with a mick method. [2] However, the saline-soaked wicks caused too much cell damage and inflammation during the wick insertion into the subcutis and the wick protocol was ultimately modified to implant dry wicks. [3] Additionally, Aukland, Fadness, and Reed introduced a modified version of the wick technique in vivo known as the "wick-in-needle" technique. [3] The wick-in-needle technique consisted of using a thin hypodermic needle with approximately a 3 mm-long side hole filled with

multifilamentous nylon wick. This modified wick technique measured the interstitial fluid pressure in rats. [3]

Dr. Helge Wiig introduced another modified in vivo wick technique that attempted to accurately measure the concentrations of different proteins in rabbits and mice. [4,5] This wick protocol consisted of using three-stranded nylon wicks. The wicks were prepared by prewashing them in acetone, ethanol and distilled water. For some experiments dry wicks were utilized. The wicks used in Dr. Wiig's experiments were inserted along with a catheter. [4] After removing the wicks, they were centrifuged in oil in order to dissociate the interstitial fluid from the wick. The successful isolation and analysis of interstitial fluid suggests that it should be possible to combine the wicks with the probes and use the wicks to accurately measure localized concentrations of various analytes around a probe. Using a probe alongside a wick in vivo would allow the recovery of the probe to be compared with the recovery of the wick.

This experiment focuses on recovering methyl orange and FITC-4000 in vitro using the wick technique. Methyl orange was chosen because it has a relatively low molecular weight and therefore should be readily absorbed by a wick. Methyl orange should also be relatively simple to quantify. FITC-4000 was chosen because it has approximately the same molecular weight as many small peptides found in vivo such as insulin. Another reason FITC-4000 was chosen is because it is widely used in research regarding microcirculation and cell permeability. [6] The structures of methyl orange and FITC-4000 are seen in figure 1 and 2 respectively. A unique protocol that used water instead of oil was attempted on FITC-4000 to determine if

the hydrophilic groups on the FITC-4000 caused FITC-4000 to dissociate from the wick more effectively in water than in oil, and therefore have a better percent recovery in water.

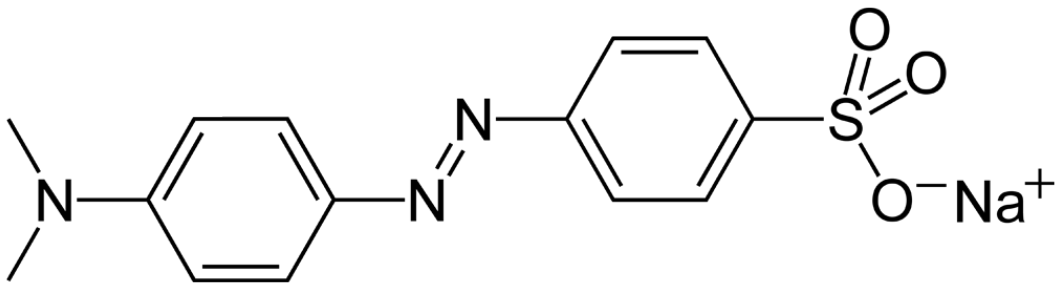

Figure 1. Structure of Methyl Orange. With a low molecular weight of 327.33 g/mol, methyl orange was the first compound chosen to undergo a percent recovery experiment in vitro. Methyl orange appears bright orange, which makes it relatively easy to extract from oil after centrifugation.

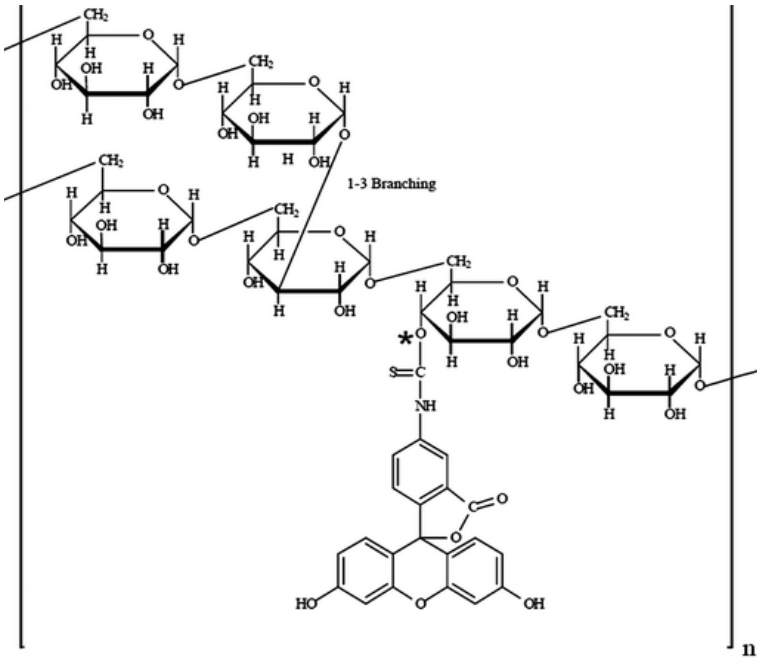

Figure 2. Structure of FITC-4000. Dextran is attached to the to the above molecule in order to create a Fluorescein isothiocyanate with a molecular weight of 4000g/mol. FITC-4000 appears green in solution. As seen above, FITC-4000 contains hydrophilic groups that may affect the ability of FITC-4000 to be extracted from the wick.

The objective in this experiment is to ultimately work up to recovering cytokines in vivo using a wick. In order to achieve this, percent recovery experiments will be performed using a wick technique in vitro. These experiments attempt to recover various analytes such as methyl orange and FITC-4000. The molecular weight of the analytes will be increased after each successful experiment in order to determine if recovering large analytes (specifically cytokines) in vivo using a specific wick technique is possible. The percent recovery of the in vitro experiments will also provide a basis for the expected percent recovery in vivo.

Methods:

Materials and Chemicals

The methyl orange and the fluorescein isothiocyanate–dextran were both prepared in HPLC water. The fluorescein isothiocyanate–dextran that was used had an average molecular weight of 4,000 g/mol. The methyl orange and the fluorescein isothiocyanate–dextran (FITC-4000) were acquired from SIGMA-ALDRICH Inc. and the HPLC water was obtained from Fisher Scientific. The wicks used were fishing line provided by Dr. Helge Wiig, University of Bergen, Norway and can be seen in figure 3 below.

Fig. 3 Visual of One of the Wicks Used in the Percent Recovery Experiments. This multifilamentous nylon wick is one of the wicks used in a percent recovery experiment.

Preparation of the Wicks

Throughout these experiments three-strand wicks (diameter 1 mm) were utilized. The wicks were cut in either 1 cm long or 3 cm long strands. The wick strands were untied at the ends. Some of the wicks were unwound. In order to

unwind the wicks one end was held together using a tweezers and the other end was twisted until the three strands that made up the wick came apart and unraveled.

Methyl Orange Saturation

A 100 μM solution of methyl orange in a 50 mL beaker was used into which the wicks were completely immersed. The wicks were never on top of each other. Multiple beakers were used in order to make sure the wicks were never interfering with each other in the same beaker. The wicks were soaked in the methyl orange for 30 minutes. The 30-minute equilibrium time was determined from the literature of other wick experiments.[2]

FITC-4000 Saturation

A similar protocol was utilized in order to saturate the wicks with FITC-4000. Everything was kept the same for the saturation of FITC-4000, even the 100 μM solution and a 30-minute equilibrium time.

Analysis of Wick Fluid

After the wicks were adequately saturated, they were placed into 2 mL centrifuge tubes. The 1 cm wicks were placed into a centrifuge tube that was filled with 1.5 mL of canola oil and the 3 cm wicks were placed into a centrifuge tube that was filled with only 1.0 mL of canola oil in order to compensate for the added volume of the longer 3 cm wicks. For some experiments with FITC-4000, water was

used instead of oil in order to maximize extraction. The centrifuge tubes consisting of the wick and oil/water were then centrifuged at 14,000 g for 5 minutes. After centrifugation, the methyl orange or FITC-4000 and the oil/water in the centrifuge tubes was aspirated out and placed in clean centrifuge tubes. These centrifuge tubes were then centrifuged for 5 minutes at 800 g. The methyl orange or FITC-4000 remaining at the bottom of the centrifuge tubes after this centrifugation was then transferred to a clean 1mL tubes. UV-Vis analysis was utilized in order to determine the absorbance of the sample at 520 nm for methyl orange and 493 nm for FITC-4000. The concentration of methyl orange or FITC-4000 in the samples was obtained by using the absorbance from the UV-Vis instrument and a calibration curve (figure 7). Once the concentration was found, the percent recovery was calculated by dividing the concentration of each sample to the original concentration.

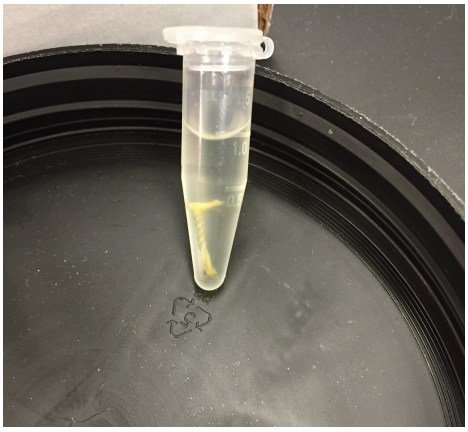

Fig. 4 Initial Setup of the Wick in the Centrifuge Tube. The wick can be seen in the bottom of the centrifuge tube. The wick has been saturated in Methyl Orange, placed in oil, and is ready for the initial centrifugation.

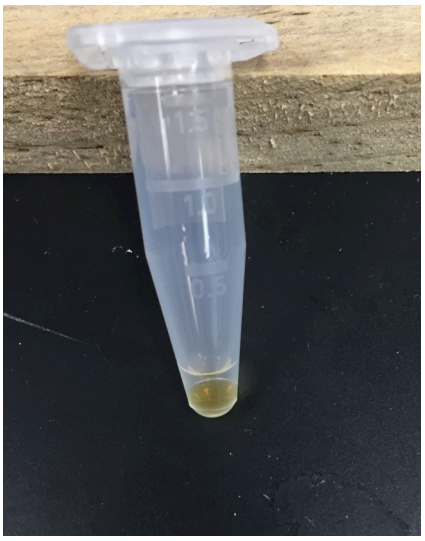

Fig. 5 Distinct Layers after Centrifugation. Two distinct layers can be seen in the centrifuge tube after the final centrifugation. The methyl orange is the lower layer and the oil is the upper layer. The methyl orange will be extracted for UV-Vis analysis.

Results:

Figure 6. shows the percent recovery of methyl orange for 1 cm unwound wicks, 3 cm unwound wicks and 3 cm wound wicks was 90.1 $\pm$ 1.7 (n=3), 90.4 $\pm$ 3.0 (n=3), and 79.8 $\pm$ 1.7 (n=3) respectively. The percent recovery was calculated by finding the ratio of the concentration of methyl orange in the wick to the concentration of methyl orange in the beaker. The 1 cm wound wicks had no data because it was not possible to keep the 1 cm wicks wound during the 30-minute soak in methyl orange or FITC-4000 without using additional materials.

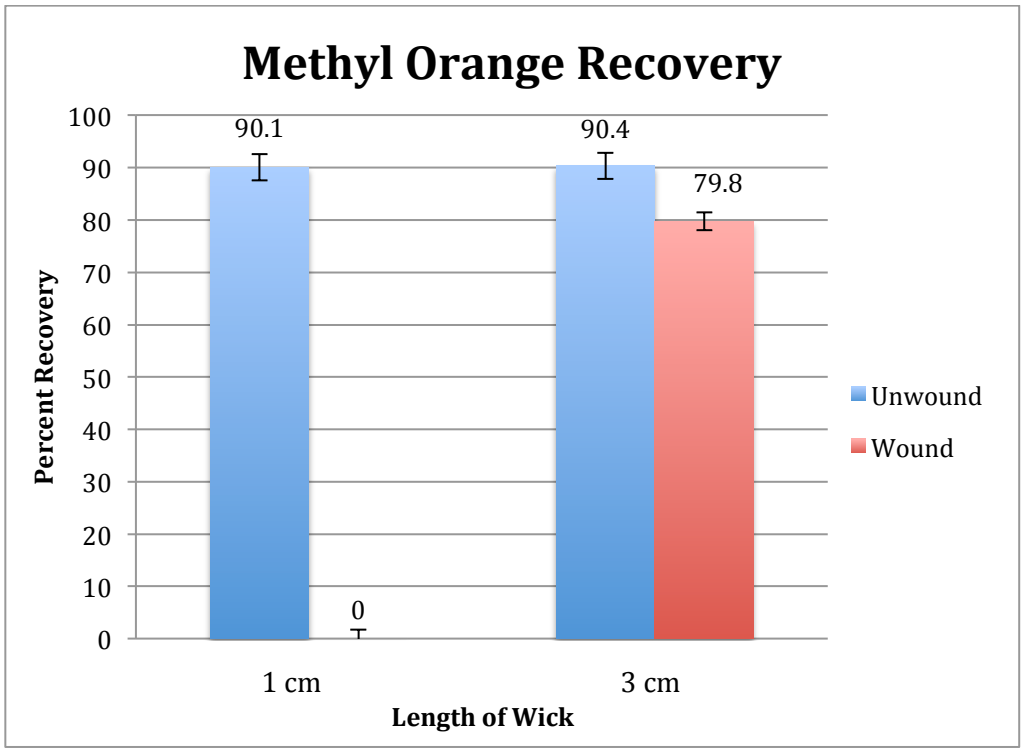

Figure 6. Methyl Orange Recovery for 1 cm and 3 cm wicks. Error bars represent the standard error of the mean (n=3).

This experiment was performed in order to learn how wick properties would affect the percent recovery. Each subsequent percent recovery experiment was performed using 1 cm unwound wicks. Additionally, each percent recovery experiment utilized a linear calibration curve of absorbance vs. concentration. An example calibration curve used in a percent recovery experiment of methyl orange is shown below in figure 7.

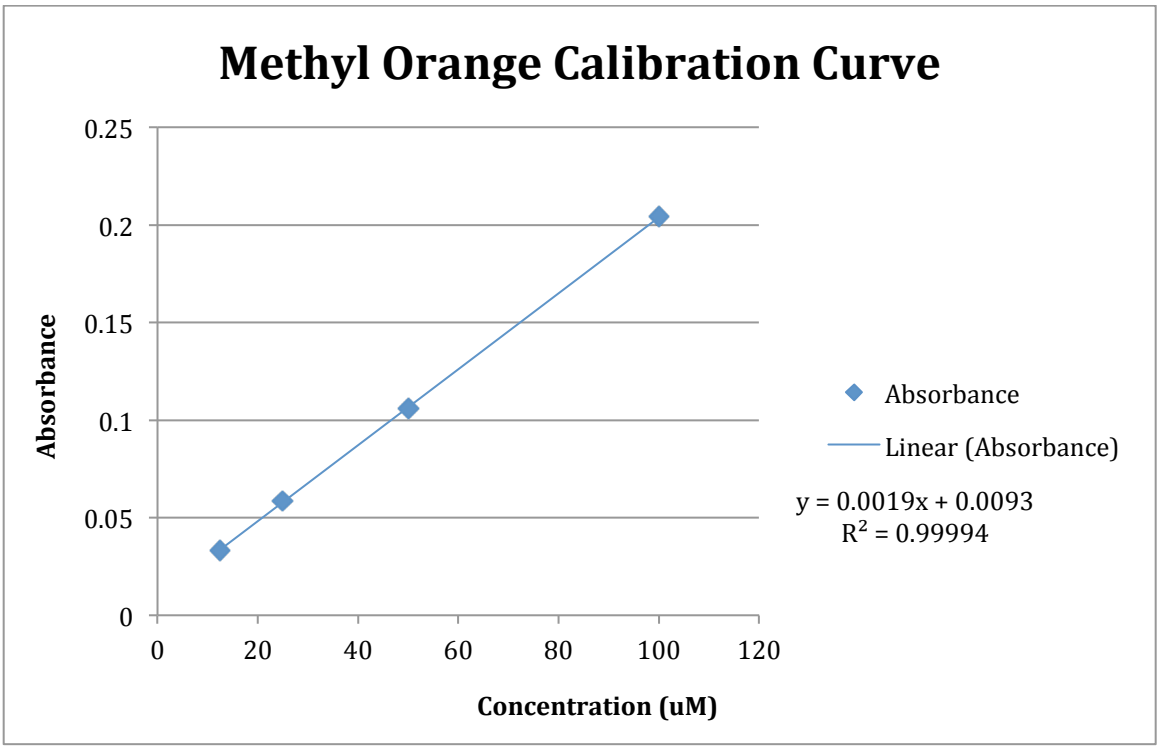

Figure 7. Calibration curve of methyl orange used to calculate concentration using absorbance values.

The calibration curves for both the methyl orange and FITC-4000 were built using standards of 100 µM, 50 µM, 25 µM, and 12.5 µM. Once the calibration curve was prepared, the methyl orange or FITC-4000 was extracted from the wick and the absorbance of the sample was determined using UV-Vis. Once the absorbance of the samples was determined, the concentration of each sample was calculated using the calibration curve. For each trial, the samples were run in triplicates. The percent recovery of methyl orange for nine separate experiments is seen in figure 8.

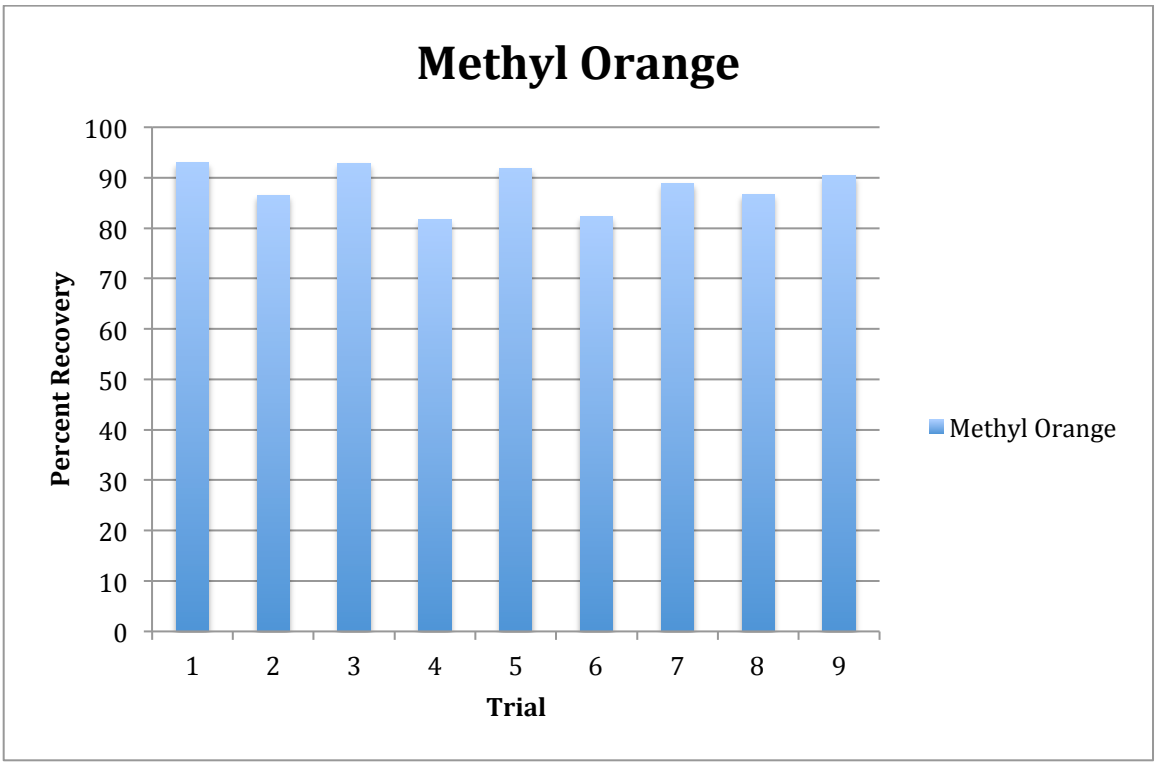

Figure 8. Percent recovery of methyl orange for nine distinct trials.

The percent recovery of methyl orange ranged between 81.7% and 93.0% for the nine trials. The percent recovery experiments with FITC-4000 were performed the exact same way as the experiments with methyl orange. Nine experiments were also performed for FITC-4000 in triplicates. The percent recovery of FITC-4000 for the nine separate trials is seen in figure 9.

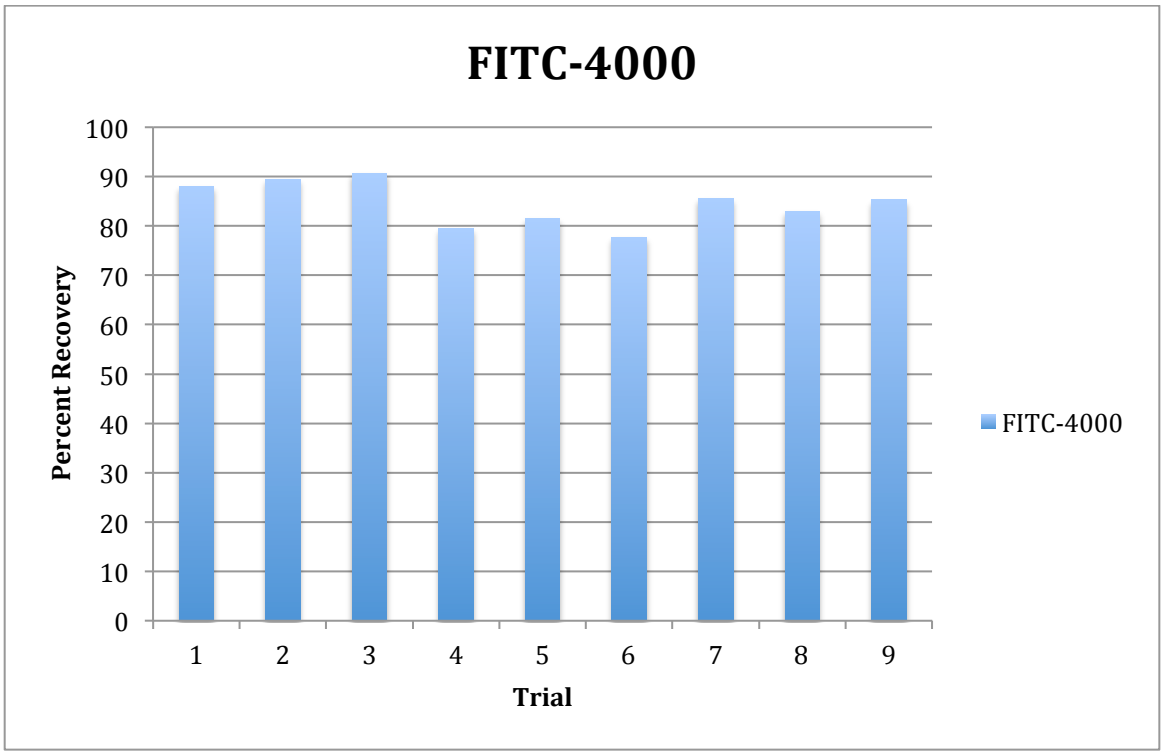

Figure 9. Percent recovery of FITC-4000 for nine seperate trials.

The percent recovery of FITC-4000 ranged between 77.7% and 90.6% for the nine experiments. There are no results from the FITC-4000 experiment that used water instead of oil because the modified protocol that used water did not yield enough sample to be analyzed. Figure 10 shows the average percent recovery for all of the trials of the methyl orange and the FITC-4000.

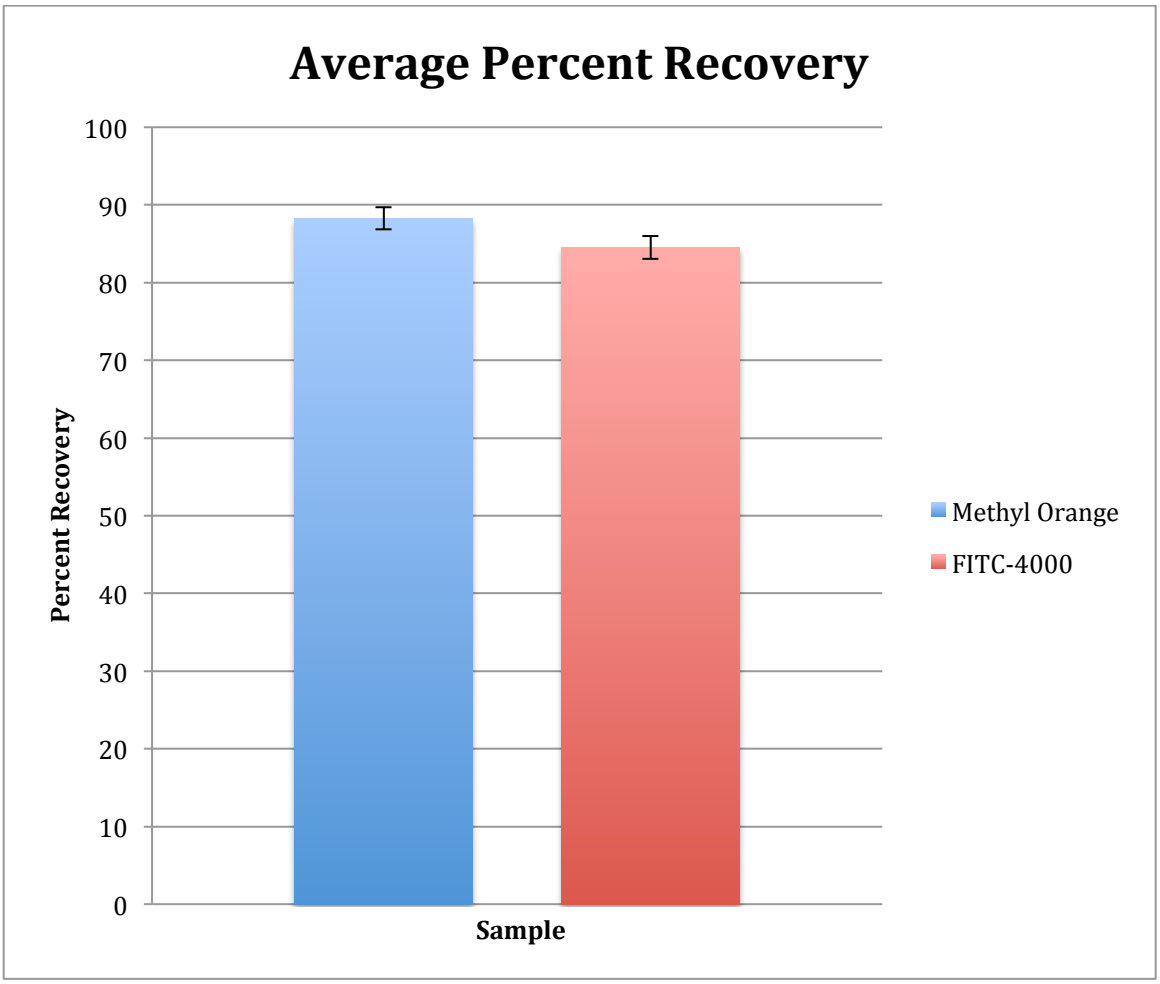

Figure 10. Average percent recovery of methyl orange and FITC-4000. Error bars represent the standard error of the mean (n=9).

It was determined that the average percent recovery of methyl orange using the wick technique was 88.3% ± 1.4% (n=9). It was also determined that the average percent recovery of FITC-4000 was 84.5% ± 1.5% (n=9).

Discussion:

These results suggest that the wick method is a dependable way to measure the concentration of methyl orange and FITC-4000 in vitro. This method seems to be rugged because it gives consistent results for the percent recovery for both samples. In the first experiment, it was determined that the unwound wicks have a higher percent recovery than the wound wicks. One plausible explanation for this is that the unwound wicks have a greater surface area compared to wound wicks of the same length. This would lead to more area for the molecules to bind which could contribute to the unwound wicks having a higher percent recovery.

These experiments also show that the percent recovery for the methyl orange was higher than the percent recovery for the FITC-4000. This could be caused by the differences in molecular weight between the two samples. Since FITC-4000 has a higher molecular weight than methyl orange, it will be more difficult for the FITC-4000 to bind the wick and therefore have less percent recovery than methyl orange. Possible explanations for this phenomenon could include less molecules of FITC-4000 being able to "fit" in the available surface area of the wick, or more attractive forces required for the larger FITC-4000 molecules to bind to the wick. These results start to suggest a trend that the higher molecular weight a sample has, the lower percent recovery will be obtained using the wick technique.

Having said that, these results are encouraging for future experiments using the wick technique because the percent recovery in these experiments was relatively high (over 80%). Future percent recovery experiments using the wick method should be done on samples with increasingly larger molecular weight in

vitro in order to make sure the wick method is effective for evaluating percent recovery in larger samples. These experiments also suggest that the wick method could ultimately be used to measure the concentrations of cytokines and other small proteins in vivo and may be able to calibrate what is happening in the living system.

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