Warfarin, a commonly used anticoagulant, is often administered to patients through feeding tubes. Recent research has revealed that there is a decrease in its bioavailability, not solely based on the drug’s ability to bind to nutrition as many had believed, but rather based on warfarin’s adsorption to the feeding tube. In this study, we further verify the interaction using ultraviolet-visible spectrotometry and provide evidence that suggests that warfarin is binding to the feeding tube. In addition, we offer data that contradicts with the prior theory that this interaction is pH dependent.
1 INTRODUCTION

Warfarin, also known commercially as Coumadin, is a commonly used clinical drug that inhibits blood clotting by hindering the production of vitamin K dependent factors. The administration of warfarin, an anticoagulant, is complicated since dosages widely fluctuate from patient to patient due to a possible variety of factors: the drug’s complex chemical properties and reactivity, patients’ genetic profiles, concentration, etc. [1-5]. It is specifically known for its potential complications with other medications, and its potential potent therapeutic effects if administered incorrectly.

Previous studies have led many to believe that the administration of Warfarin through feeding tubes should occur 1-2 hours before and after nutrition injections due to the current understanding that the warfarin binds to the nutrition causing the patient to receive an incomplete dosage. This theory has led to drastic changes in the way warfarin is administered; yet, even with these modifications in many hospitals around the country, the bioavailability of dissolved warfarin when administered through feedings tubes continues to vary drastically in patients even when enteral nutrition formulas are held for hours prior to and after drug delivery.

Recently, Klang et al. proposed that warfarin, in addition to binding to the nutrition, was adsorbing to patients’ feeding tubes. Their study investigated the effect of pH on warfarin adhering to two different types of feeding tubes-Entriflex and Dobb Hoff-used at Memorial Sloan Kettering Hospital in New York City. To mimic the physiological states of the human body and the path by which the feeding tube passes through, the stomach (pH \approx 1.5) and jejunum (pH \approx 6.5) were imitated by using hydrochloric acid and sodium hydroxide. Specific amounts of feeding tube were then added to each of the 200 mL solutions (the amount of fluid in the stomach) at normal body temperature (37\, ^\circ\, \text{Celsius}). The change in concentration of Warfarin was measured using High Performance Liquid Chromatography (HPLC), and their results suggested that the warfarin bioavailability drastically changed in the low pH environments compared to the high pH environments. This was especially the case in the presence of feeding tube where the concentration decreased by approximately 50\% in one hour; moreover, a small percentage warfarin was recovered when the feeding tube was removed from the low pH and placed into a newly made high pH solution. In addition to
this, the researchers also created an in vitro model of the interaction that pumped warfarin through a feeding tube and into a synthetic stomach. The results were quite similar [6]. Unlike past experiments, which analyzed this phenomenon in impractical pH environments between 7 and 8 [7], these two recent studies accurately simulated the molecular environments where warfarin is absorbed and where the interaction would take place by using lower pH solutions. Thus, the results of Klang et al. provide a novel explanation for why patients when receiving dissolved Warfarin through a feeding tube are not obtaining their full dosages.

In this experiment, we further considered this alternative explanation for warfarin’s decline in bioavailability when administered through feeding tubes. To begin, the molar extinction coefficients (ε) for both high (~6.5) and low (~1.5) pH environments were determined by measuring warfarin’s absorbance at set concentrations. We then confirmed that warfarin was stable at low concentrations in both high and low pH environments; we also noticed that warfarin precipitated out of low pH solutions at high concentrations, which is an observation that was not mentioned in prior studies and thus should investigated in the future. After employing a variation on Klang et al.’s two techniques, we confirmed that warfarin was interacting with DOBBHOFF Feeding Tubes; on the other hand, our results provided evidence for the opposite pH effect occurring. In high pH solutions, approximately 82% of warfarin was lost, in comparison to 59% in low pH solutions. Furthermore, recovery of the lost concentration in both pH solutions was limited.

Modeling warfarin with fluorescein, a fluorescent dye that has a similar chemical composition (Figure 1), our study demonstrated that a very specific interaction is occurring. While a layer of fluorescein, remained on the feeding tube after it had been drained, it, unlike warfarin, was easily rinsed out as seen in fluorescence photographs taken from under a microscope. This suggests further that warfarin is not only adhering to or becoming trapped in any porous locations in the tube, but presumably is binding to the feeding tube. In the last part of our study, we made some minor assumptions in order to quantify the interaction and estimate a low value for the equilibrium constant (K~137519.9). Overall, this article further strengthens, at the same time as it questions, the arguments made by Klang et al. in regards to the interaction between warfarin and feeding tubes.
The chemical compositions of warfarin (A) and fluorescein (B) are quite similar. Both substances have benzenes attached to ether rings, ketones and phenols. In addition, to comparable structures, the fluorescent characteristic of fluorescein in the presence of ultra-violet light made it a good model to further probe this interaction in this experiment.

## 2 EXPERIMENTAL

### 2.1. Materials

Warfarin Sodium (98% purity; TCI America, USA); 1M Hydrochloric Acid (Fisher Scientific, USA); DOBBHOFF Dual Port Feeding Tube with FLOW-THROUGH Stylet (TYCO Healthcare, USA); [Distilled water system] and Fluorscein were employed in this in-vitro study.

### 2.2. Measuring the bioavailability of warfarin with ultraviolet-visible spectrometry

Quartz cuvettes

(forget to ask company name/ equipment information)

### 2.3. Preparation of warfarin solutions
The warfarin arrived in powder form. As required for each experiment, warfarin was prepared in clustered solutions, ranging from a concentration of 0.0005 M to 0.00000625 M, of either distilled water or hydrochloric acid (pH of 1.5). All solution concentrations produced were measured by means of ultraviolet-visible spectrometry at an average absorbance value of 304.76 nm (±1.94) and then calculated based on the molar extinction coefficient attained from the characterization of the absorbance standard curve. In addition to recording the absorbance at the beginning of every experiment, each solution was established to be the same by also recording the absorbance at the end of the experiment, unless having been exposed to the DOBBHOFF feeding tube. In this way, it was confirmed that the interaction loss in warfarin’s concentration was on account of the feeding tube, rather than some extraneous factor that had not been identified (e.g., degradation).

2.4. Characterization of the standard absorbance curve for warfarin

To determine the concentration of each warfarin solution produced during the experiment, two sets of standard samples of known concentration (0.0001, 0.00005, 0.000025, 0.0000125, 0.00000625) were prepared, one in water and one in hydrochloric acid; the absorbance was then measured for each sample. Using Microsoft Excel (version 12.1.5), an absorbance calibration curve was generated for both distilled water and hydrochloric acid by using linear regression. Regression analysis of the plot yielded $R^2$ values for both types of solutions.

2.5. Preparation and utilization of feeding tube

The metal wires were removed and the end portals were severed off of the DOBBHOFF feeding tubes. Feeding tubes were cut into pieces approximately 75 centimeter long; this length was necessary in order to have sizeable amounts of solution to fill the quartz cuvette. By inserting a 2mL pipette into one end of the feeding tube and by placing the other end into the solution, we were able to draw up the solution into the feeding tube. On each end, clamps were used to seal in the solution. The amount of time the warfarin solution spent in feeding tubes varied from fifteen minutes to over an hour. In specific
circumstances, the same method to draw up the warfarin solution was used to draw up water or fluorescein. Water was particularly used to rinse out any warfarin, which was neither bound nor trapped, and yet, remained in the tube.

2.6. **Modeling the bioavailability of warfarin with fluorescein**

Fluorescein was utilized by two techniques to further probe the interaction between warfarin and the feeding tube due to its similar functional groups and structure. Prior to this study, there were no known experiments, which have used fluorescein for this purpose. In our experiment, the UV absorbance was 489.71 nm. The desired solution of fluorescein generated was to be comparable to that of warfarin. As with warfarin, its absorbance was measured before and after an hour had passed, in addition to after it had been in the feeding tube for an hour. To calculate the concentration of fluorescein, we used a molar extinction coefficient of 80,000 M\(^{-1}\) cm\(^{-1}\), which was based on the literature read about fluorescein [8-10]. In addition to studying its absorbance, the tube, in which the fluorescein had been left in for an hour was cut into two pieces to be observed under a microscope. While one piece was left unhandled, the other was rinsed once with water. A camera hooked up to the microscope was then used to capture the difference in fluorescence between the tube that had been washed and the one that had not been washed.

2.7. **Quantifying the interaction between warfarin and feeding tube**

A batch of approximately 0.0008 M warfarin solution was created in order to quantify the interaction between warfarin and feeding tube. This solution was then diluted by 10 using hydrochloric acid, into four different ~0.00008 M solutions. These solutions, one after another, were then left in the same 75 cm feeding tube for 1 hour each. Each solution was removed after an hour before the next was introduced and the absorbance value was recorded for each of the solutions removed as well as for the solutions placed in the tubes. We were able to monitor if a layer of warfarin was in fact forming due to affinity or becoming trapped on the inside surface of the feeding tube. The absorbance values
before and after an hour, as well as the weight of tubing before and after, were then used to calculate a crude physical model of the equilibrium constant, $K$.

3 RESULTS

3.1 Determining Warfarin’s Molar Extinction Coefficient

As described in section 2.4., a warfarin standard absorbance curve was generated from samples of known concentrations in both water and hydrochloric acid (Graph 1). The molar extinction coefficient for warfarin in water was calculated to be 12,784 M$^{-1}$ cm$^{-1}$ ($R^2=0.99995$). The molar extinction coefficient for warfarin in hydrochloric acid was calculated to be 8,519 M$^{-1}$ cm$^{-1}$ ($R^2=0.99993$). These coefficients were then used to calculate the concentration for any absorbance value obtained from this point on.

### Warfarin Standard Absorbance Curve

In this graph, standard concentrations of warfarin (0.0001, 0.00005, 0.000025, 0.0000125, 0.00000625) were plotted in relation to their observed absorbance values in both water (green) and hydrochloric acid (purple).

3.2 At Low Concentrations Warfarin is Stable
Early on in the study, it became clear that large concentrations of warfarin were not easily soluble in HCL solutions. In order to avoid warfarin precipitating out of solution, it was dissolved first in water and then HCL was added after to adjust the pH. Another early observation was that our spectrometry detected two peaks of absorbance for warfarin in the low pH solution, while it only detected one peak for warfarin in the high pH solution. With these two discrepancies in mind, we decided to inquire whether warfarin concentrations were stable at low concentrations.

Our results suggest that warfarin is stable at concentrations below 0.0005 in both high and low pH environments (Table 1,2). The stability of warfarin was studied for at least one hour. There was modest variation over time in terms of concentration. For the 0.0005 M warfarin water based solution the standard deviation across the samples was 0.0000016. For the 0.00005 M warfarin water based solution the standard deviation across the samples was 0.0000012. For the 0.00005 M warfarin hydrochloric acid based solution the standard deviation across the samples was 0.0000012.

<table>
<thead>
<tr>
<th>TIME (MINUTES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>O.0005</td>
</tr>
<tr>
<td>O.00005</td>
</tr>
</tbody>
</table>

Table 1: Stability of Warfarin in a Water Based Solution

<table>
<thead>
<tr>
<th>TIME (MINUTES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<tr>
<td>O.00005</td>
</tr>
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</table>

Table 2: Stability of Warfarin in a Hydrochloric Acid Based Solution
3.3 Loss of Warfarin Occurs Upon Exposure to Dobb Hoff Feeding Tube
Interaction Between Warfarin and DOBBHOFF Feeding Tube

Our results undoubtedly support the argument that warfarin interacts with feeding tubes. As shown in Graph 2, when warfarin is in a low or high pH solution and introduced to feeding tube for one hour the concentration drops drastically. The percent of warfarin lost was 82.1% in water and was 58.5% in HCl. The original solution, which remained in a closed Erlenmeyer flask for one hour, did not display a significant loss in concentration, but rather as expected was very closed to the original recorded concentration.

Graph 2: Loss in Warfarin Concentration
In this graph, the interaction between warfarin and feeding tube is illustrated. The original concentration remained the same in both water and HCl after one hour; however, when the original concentration was inserted into the feeding tube for one hour, the concentration declined. The purple bars represent the wafarin in high pH (~6.5), and the green bars represent warfarin in a low pH (~1.5). *Note: The concentration of warfarin in HCl represents the average of two different trials.
In addition to examining the lessening in concentration of warfarin in feeding tube, we also investigated if the warfarin could be recovered from the feeding tube. In the first attempt of rinsing the tube out with water about 15.4% of the warfarin lost was recovered. After leaving water in the same tube for 5 hours, another 13.9% of what remained lost was recovered from the feeding tube.

3.4 Probing The Interaction With Warfarin Model: Fluorescein

After leaving Fluorescein in feeding tube for one hour there was only a 20.4% loss in concentration (Graph 3). This in comparison to the percent of warfarin lost after one hour is exceedingly low. This suggests that fluorescein does not have the same affinity as warfarin for the feeding tube.

Graph 3: Loss in Fluorescein Concentration in Feeding Tube
Each bar represents the same fluorescein solution under different conditions: after one hour and after one hour in a feeding tube.

In addition, the feeding tube was cut into two pieces and observed under ultraviolet light. One piece was not washed (Figure 2A), while the other was rinsed once with water (Figure 2B). Under close observation, an orange ring is visible in the tube’s lumen in Figure 2A; however, in Figure 2B, there is no fluorescein coating the lumen. As a result, we can suggest that the small amount of concentration lost in Graph 3 is on account of fluorescein adsorbing to the surface of the feeding tube.
3.5 Quantifying the Interaction Between Warfarin and the Feeding Tube

In order to learn more about the interaction taking place, this part of the study, as explained in 2.7, added continual concentrations to the same tube and observed if the percent of warfarin lost decreased over time. Specifically, this experiment attempted to figure out the lumen’s saturation point. Below is an equilibrium diagram, based off of our results:

<table>
<thead>
<tr>
<th>Warfarin (Aq)</th>
<th>Warfarin (Lost)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000018 M</td>
<td>⇋</td>
</tr>
<tr>
<td>0.000023 M</td>
<td>⇋</td>
</tr>
<tr>
<td>0.000035 M</td>
<td>⇋</td>
</tr>
<tr>
<td>0.000038 M</td>
<td>⇋</td>
</tr>
</tbody>
</table>

This data was then used to calculate the molar concentration (5.28 mol), as well as, the equilibrium constant (137519.91 K). In Graph 4, it seems as though the warfarin concentration binding to the feeding tube is beginning to plateau, which means that our proposed K is a lower estimate than what it really is. It also becomes aware from plotting both the warfarin lost and the warfarin left in the aqueous solution that if the same solution was inserted approximately two times more, the lumen would have reached saturation.
Graph 3: Estimating K and Finding the Lumen’s Saturation Point
In this graph the purple circles illustrate the concentration of warfarin left in the solution after one hour in the feeding tube and the green circles illustrate the calculated the warfarin lost.

3 DISCUSSION

In this study, it has been demonstrated that an interaction between DOBBHOFF Feeding Tubes and warfarin is feasible. Furthermore, our data suggests that the warfarin is not physically trapped in the lumen or sticking to the lumen by means of an electrostatic field. Warfarin seems to have an affinity for Hydromer, the coating that makes up the lumen of DOBBHOFF Feeding Tubes, and is thus binding to the surface of the feeding tube causing a loss in concentration. Based on the literature, Hydromer is a lubricious material, which is mainly generated by combining polyvinylpyrrolidone (Figure 3A) and other various isocyanate prepolymers [11]. Polyvinylpyrrolidone is a strong polar molecule; yet, warfarin is considered to be non-polar, and thus this complicates our results. On the other hand, in a recent patent titled, “Coating for biomedical devices,” the authors alluded to other patents where a blend of polyurethane (Figure 3B) and a different hydrophilic polymer is commonly used to coat feeding tubes.
In other words, it is hard to conclude what the chemical structure of hydromer might be, and therefore, what functional groups might be interacting with the functional groups of warfarin.

![Chemical Structure of Polyvinylpyrrolidone (A) and Poluurethane (B)]

Another interesting finding in our study was that the loss in warfarin concentration was greater in high pH environments than in low pH environments. In addition to this, in low pH solutions the absorbance curve was a double peak rather than a single peak as seen in high pH solutions. As shown in Nicholls et al., warfarin’s structure can change depending on the environment it is exposed to (Figure 4)[1]. This property of

![The many structures of warfarin](image)
warfarin to may explain why there is a difference in warfarin concentration as well as absorbance curves; however, this difference needs to be addressed when administering warfarin to patients, and further studied.

Moreover, by modeling warfarin with fluorescein, we were able to demonstrate how other substances adhere to the feeding tube. As seen with fluorescein, there can be minor concentration lost when passed through the feeding tube (20.4%); however, this is insignificant compared to the 50% or greater concentration of warfarin lost. Fluorescein also was easily removed from the feeding tube as seen with fluorescence photography, while the warfarin lost after being passed through a feeding tube was barely recovered. This further suggests that the warfarin is binding to the feeding tube. Based on the functional groups of warfarin alone, a hydrophobic effect between warfarin and the lumen should be investigated in the future.

The final part of our study, which quantified the interaction, was mainly to elucidate whether or not the lumen can be saturated and other properties of the interaction. The final trial was treated as if the reaction had reached equilibrium in order to obtain the equilibrium constant (K). The molar concentration and K values are reasonable since the tube was about 75 cm in length and thus contained a decent amount of fluid and surface area. Furthermore, in graphing the concentration from each aqueous solution, we can infer from the curves shape that the concentration will eventually plateau; however, this is also based on the assumption that warfarin will create a layer on the lumen rather than starting to bind to other warfarin molecules due to some dipole moment. Overall, it would still be useful to redo this aspect of the experiment for more trials; from the data thus far, it could be assumed that the concentration should plateau in 6-8 trials.

4 CONCLUSION

The interactions with warfarin need to be further examined. As a drug that is widely used across the country, it is essential to understand the ways in administration can hinder the bioavailability of warfarin. Furthermore, this is one of many drugs that are administered to patients through feeding tubes. Based on these results, warfarin should be administered through feeding tubes in high rather than low pH solutions. While this
contradicts the recent study by Klang et al., they did not particularly consider that the warfarin would bind to the outside of the feeding tube when cut into small pieces. This is only the first step in understanding how warfarin binds to the lubricious lumen of feeding tubes.

REFERENCES