DEVELOPMENT, CONSTRUCTION AND VALIDATION OF A TWO-COMPARTMENT ANALYTICAL INSTRUMENT FOR USE AS AN ALTERNATIVE DISSOLUTION METHOD FOR PHARMACEUTICAL TESTING

By
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Submitted to the graduate degree program in Pharmaceutical Chemistry and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science.

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Abstract

The purpose of this research was to develop, construct and validate a two-compartment dissolution testing instrument as an alternative test methodology to the current standard USP apparatus II system. The current USP apparatus II system was developed in the 1970's and has known defects in its design related to fluid dynamics and bio relevance. This two-compartment dissolution instrument eliminates the issues of fluid dynamic mixing, use of biorelevant media levels and provides clinically relevant data. The two-compartment dissolution instrument was tested in conjunction with GastroPlus simulations to mimic a human model with drug absorbance set to zero. This instrument was able to produce similar gastric and duodenum amount recovery profiles from GastroPlus for the two compounds tested.

Acknowledgements:

I would like to thank my advisors, professor Dr Jeffrey Krise and Dr John Chung, for their patience, understanding and guidance through this research project. Without their guidance and knowledge this project would not have evolved into the instrument seen today.

I also would like to thank Amgen for the support to pursue this degree through the University of Kansas master's program. It is great to have the full support and backing of a company devoted to bettering the science of delivering better products to our patients.

I am thankful to the University of Kansas to offer this distance master's program. This whole experience has greatly developed my skills and knowledge in my day to day job. From degradation pathways, to analyzing results to why clinical trials are set up in a specific way. This program has greatly helped in obtaining the position I am in today and where I will go in the future.

Lastly, I want to praise my wife and daughter for their support while I pursued this degree. For all the weeknights and weekends that I missed with the family as I worked on this degree, they never once waivered in their full support. I am truly blessed to have them in my life.

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Chapter 1. Introduction

1.1 Pharmaceutical Testing Overview for Solid Oral Dosage Units

During the lifecycle of a solid oral dosage unit, such as a capsule or a tablet, in the pharmaceutical industry there are multiple tests used to ensure the safety, efficacy and quality of the drug product. These tests are applied to detect differences from one lot manufactured to the next. The tests are applied to monitor the drug product performance in different temperatures and humidity conditions over time. This thesis is focused on one specific test, the dissolution test.

The dissolution test is important as it is the only analytical test used that monitors the disintegration of the oral solid dosage and the dissolution of the active pharmaceutical ingredient in media. The dissolution of the active pharmaceutical ingredient in media over time is known as the dissolution rate. The dissolution rate is important because it can predict whether the active pharmaceutical ingredient will be effective or not.[1] The dissolution rate can also be used to predict serious situations to avoid when taking the oral solid dosage. For example, Palladone XL (hydromorphone hydrochloride), which is designed to be a modified release dosage form which means it releases the active pharmaceutical ingredient slowly over time in the digestive system, resulted in a six-fold increase in blood levels when ingested with alcohol.[2] The presence of the alcohol interfered with the tablets ability to slowly release the drug over time. This is known as alcohol dose dumping,[2] and could result in death.

The dissolution test is used as a quality control (QC) test,[3-4] as a tool for developing a drug product formulation,[5] and for In Vitro In Vivo Correlation (IVIVC) studies or bioequivalence (BE) studies.[3, 6-9] The test is conducted in one of seven United States

Pharmacopeia (USP) approved dissolution apparatuses.[10] The seven USP approved dissolution systems are:

USP Apparatus 1 – Rotating Basket

USP Apparatus 2 – Rotating Paddle

USP Apparatus 3 – Reciprocating Cylinder

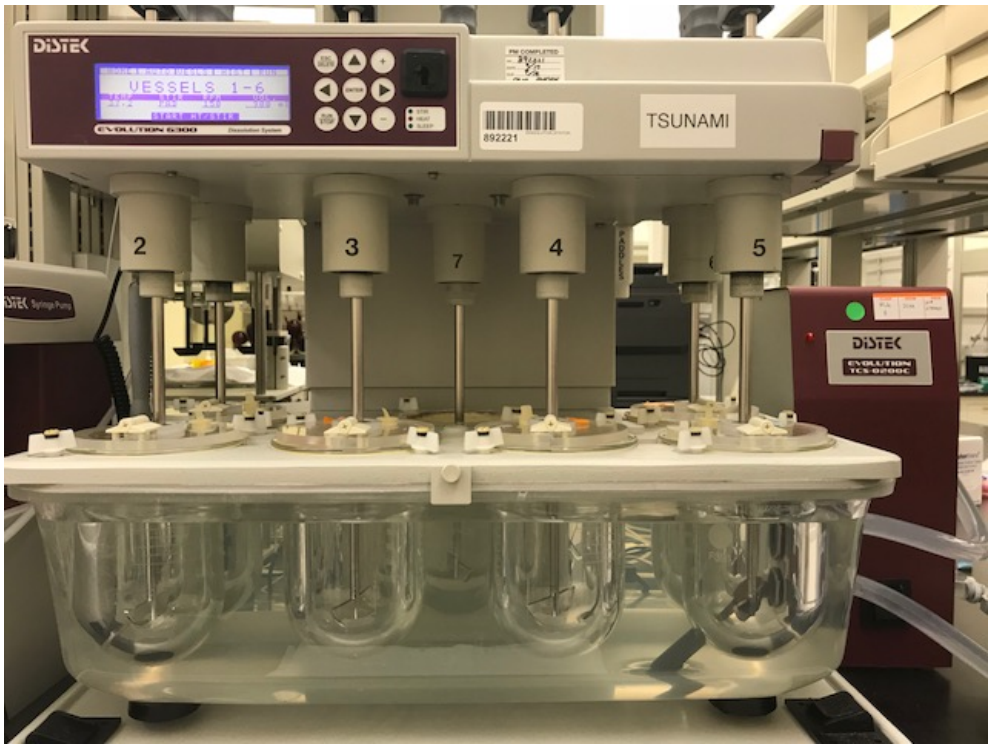
USP Apparatus 4 – Flow Through Cell

USP Apparatus 5 – Paddle over Disk – modification of Apparatus 2

USP Apparatus 6 – Rotating Cylinder – modification of Apparatus 1

USP Apparatus 7 – Reciprocating Holder – modification of Apparatus 3

The most commonly used dissolution apparatus is the USP Apparatus 2 system. The system was first introduced in the 1970's.[11] A picture representing a current design of the system is seen in Picture 1.



Picture 1: Distek Evolution 6300 Paddle Configuration, USP Apparatus 2 System

The system consists of at least six one-liter round bottom vessels that are suspended in a hot water bath. The hot water bath is kept at a steady temperature of 37.0 +/- 0.5 °C[9] (97.7 °F to 99.5 °F). The vessels are filled with 500 to 1000 mL of media as required per the analytical method being followed, though the most common volume used is 900 mL.[12] A paddle is suspended at a prescribed depth of the vessel and used to mix the media at a set speed, typically between 50 to 100 rotations per minute or RPM.[12] Samples are withdrawn at certain times and analyzed for the percent amount of drug present in that aliquot. The results are plotted as percent released versus time. An example is shown in Figure 1 below.

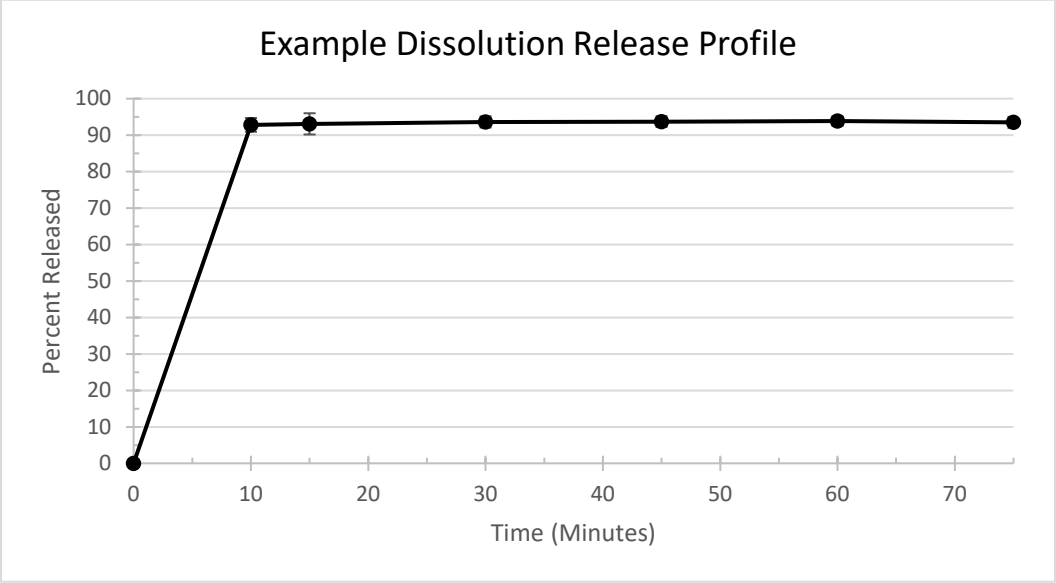


Figure 1: Tablet USP Apparatus II Dissolution Plot Example

1.2 Quality Control Test Versus IVIVC

There are two main different uses for the dissolution test. The two uses are for quality control testing of the oral solid dosage unit and IVIVC testing. The quality control test is developed and used to detect differences in the oral solid dosage unit from lot to lot or over time. The IVIVC test is developed and used to predict the oral solid dosages performance in the body using lab instrumentation.

The first use is the quality control (QC) test. The QC test is properly developed and validated[11-13] to aid in drug product development[5] as well as perform release and stability testing of the drug product.[4] The QC test is the only analytical test that is used to detect the disintegration and dissolution performance of the oral solid dosage from one lot of material to the next. The QC dissolution test is used to test the oral solid dosage from research through commercial manufacturing.

The QC dissolution method is developed alongside formulation development of the drug product. The dissolution test monitors the disintegration of the drug product as well as the dissolution of the active pharmaceutical ingredient in a selected media. The media is selected based off knowledge of where in the digestive system the oral solid dosage is intended to disintegrate. For example, if the oral solid dosage is intended to disintegrate in the stomach, then a gastric media is used for the dissolution test. From there the test is developed using guidelines from the USP on development of the test (apparatus to use, running parameters to begin with).

Ideally the test is developed with multiple lots of the oral solid dosage that has been prepared slightly differently by the formulation scientists. For example, one lot of tablets could be pressed harder, another could have a larger layer of coating applied. Both of which would modify the disintegration parameters of the oral solid dosage and thus impact the release of the active pharmaceutical ingredient. This helps the formulation scientists learn what specific steps in the manufacturing process are critical to the performance of the of the oral solid dosage unit.

The dissolution QC test is also used to study the oral solid dosage performance over time at certain temperatures and humidity conditions. These studies are known as stability studies.[4] The stability study is conducted by putting the drug product in a container, or can be open dish, and placed in qualified chambers that maintain a certain temperature and humidity. An example

of a stability study design is shown in Table 1 below. The dissolution test will be used to compare results from the different conditions as well as monitor changes in the release profile in each condition over time. This means the 24-month release profile will be compared to the earlier time point release profiles. These are usually plotted together to better detect any trends or changes in the release profiles.

Table 1: Stability Study Design Example for a Single Lot of Drug Product in 30ct HDPE Bottle

Condition	0 month	1 Month	3 Month	6 Month	9 Month	12 Month	24 Month
5 °C		Test	Test	Test	Test	Test	
30 °C/ 65% RH	Test	Test	Test	Test	Test	Test	Test
40 °C/ 75% RH		Test	Test	Test			

The quality control test is developed to create a strong reproducible dissolution release profile for an oral solid dosage unit. The IVIVC method takes a release profile calculated from clinical data and creates a lab dissolution method that will create the same profile. The reason for this is to potentially apply for a biowaiver which can save time and money for the pharmaceutical industry.[6-9]

The IVIVC test starts with the clinical release profile and works backwards to establish the dissolution instrument running parameters, media, etc. Development in the USP Apparatus 2 system will focus primarily on varying the paddle speed and the composition of the dissolution medium. Paddle speeds could be dropped below the normal 50 RPM to decrease agitation which slows the disintegration rate of the oral solid dosage and there by decrease the release of the active pharmaceutical ingredient.

The IVIVC dissolution media may deviate from the QC test to a media that is more physiologically relevant. These media incorporate the use of salts and enzymes as well as can

mimic the fed and fasted stated of a typical adult human.[14-18] The media is a fantastic example of matching the true physiological model, however, it has a few disadvantages which keep it from being used routinely in the QC dissolution test.

First, the media may have an expiry of a few hours which can impact how the test is conducted. Next, the matrix of the media is much more complex, and the simple UV standalone system may not be able to be used due to interference from the media matrix. Lastly, there are new issues that arise for the analytical determination on the HPLC as well. Media matrix peaks may interfere with the active pharmaceutical peak. The sample solutions may need to be further filtered prior to injection on the HPLC. With each filtration step the repercussions to the active pharmaceutical ingredient must be closely monitored and tested.

Once the IVIVC method is developed and validated it can be used to show bioequivalence between different formulations which can eliminate the need for costly clinical trials and reduce the time to get the drugs to patients. This is completed by testing the new formulation and the original formulation side by side in three different media using the dissolution parameters validated in the IVIVC test. The release profiles are compared between the two formulations and the f2 statistical test is used to show equivalence between the profiles. If this is met, again, this would eliminate the need to perform extra costly and time-consuming clinical trials allowing the new formulation to get to patients faster.

In the end, the IVIVC method once developed and validated will, in most cases, be very different when compared to the QC method due to the changes in the USP Apparatus system operating parameters and the media.[7] In this research, there will be a focus to combine these two methods into a single test using a completely different system that was developed to better illustrate the fluid levels and stresses on the solid oral dosage form as seen In Vivo. However, to

understand this research fully, a basic overview of the human physiological digestive system is needed.

1.3 Human Physiological Digestive Basic Overview

This section focuses on the parts of the human fasted digestive state relevant to the solid oral dose disintegration and dissolution in the stomach and follow the drug as it passes to and through the duodenum. The mouth, intestines and colon will not be covered in this research. The focus of this research was centered on immediate release drugs in a fasted human model. An immediate release drug for purposes of this research will be defined as a drug that rapidly (under 20 minutes) releases the API from the selected oral dosage form in the stomach media.

The stomach is the organ in the body where most food digestion occurs.[19] When in the fasted state the stomach is not actively digesting as food is not present in the stomach. In the fasted state the stomach still carries a small volume of gastric media. The media volume in the stomach is not static. This means that we swallow saliva and the stomach will secrete a small amount of stomach juices in the fasted stated which add to the volume of media present in the stomach. As these fluids are added to the stomach, the stomach also passes a small volume of the gastric media to the duodenum chamber to maintain a steady state volume. The fluids in both the stomach and the duodenum are complex with salts and enzymes being present in varying degrees throughout the digestive cycles.

With the brief overview of the human physiological model being used in this research, the focus now is on the issues with the current USP Apparatus 2 system to understand how this research seeks to eliminate these key issues.

1.4 Problems with the USP Apparatus II System

Over the years there have been several problems highlighted with the USP dissolution apparatus 2 system. These problems vary from fluid dynamics within the vessel, incorrect volumes being used, and that the dissolution test is in a single compartment. With each of these issues noted there have been multiple attempts to solve them and implement changes to the dissolution testing.

1.4.1 Fluid Dynamics of the Dissolution Vessel

The dissolution vessel is a one-liter round bottom vessel with typically 900 mL of media present. A paddle is used for mixing the media during the test and it is positioned per the USP standards.[10] The paddle spins at 50, 75 or 100 rotations per minute (RPM). See Figure 2 from Bing Wang et al for a standard depiction of a USP apparatus II dissolution vessel.[20]

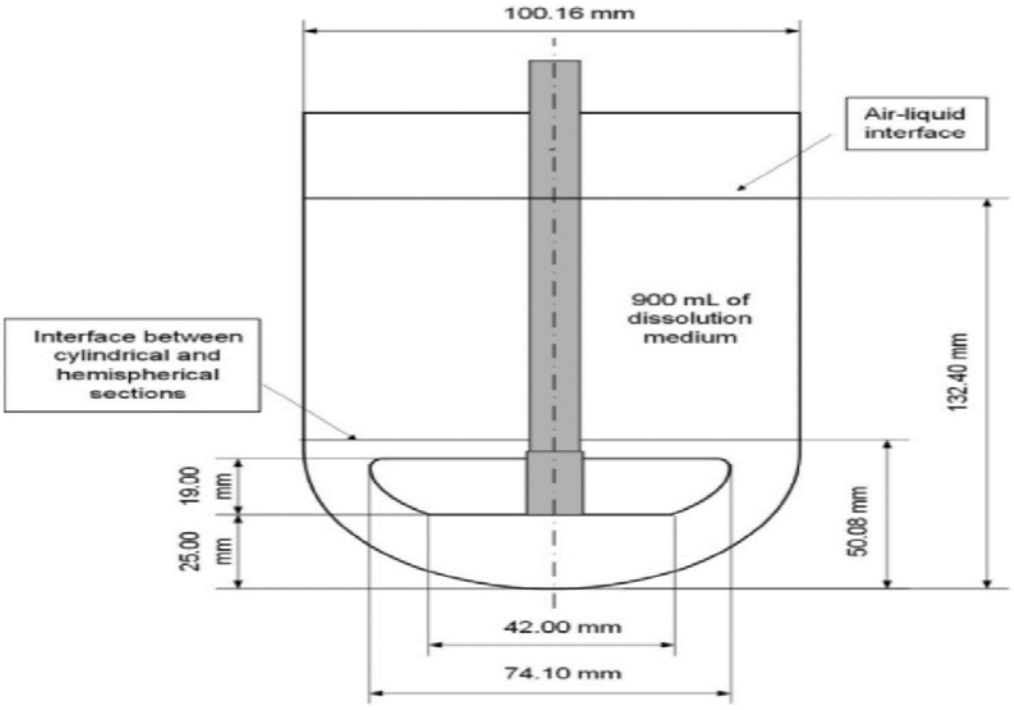


Figure 2: USP Apparatus II Dissolution Vessel[20]

The design of the dissolution vessel is set up so that when a tablet is added to the media it sinks to the bottom of the vessel and centers right under the center of the paddle. The issue with this is shown in Figure 3. This figure depicts that centered directly under the paddle shaft is a dead zone for fluid flow or agitation depicted by the blue color. As there is little to no fluid flow the boundary layer is relatively large. Following the Nernst Bruner equation this will result in a smaller dissolution rate that is a result of a design issue with the instrument.

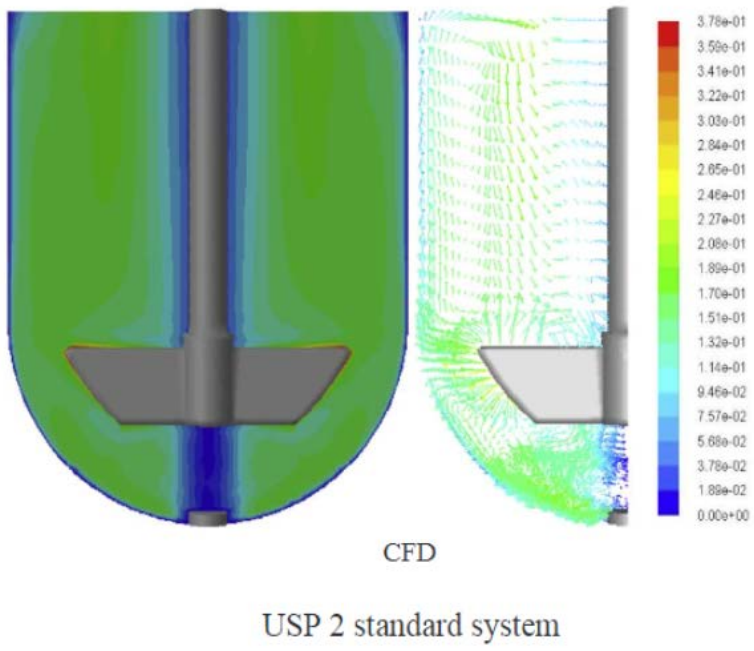
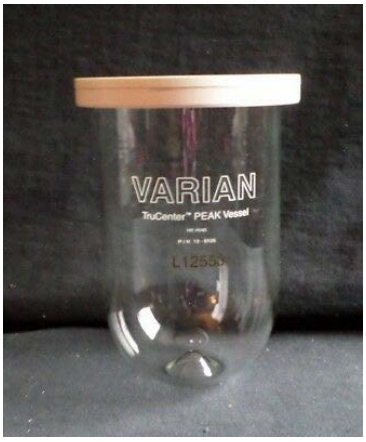


Figure 3: Fluid Velocity Contours of the USP Dissolution Apparatus II Vessel with Tablet[20]

This phenomena of the dead zone beneath the paddle also plays a major role in achieving a complete release profile when the dosage form breaks apart but forms a cone of excipients at the bottom of the vessel directly below the paddle. This is known as coning or mounding. The excipients act as an additional barrier between the drug and the media. This is an exaggerated added artifact for the boundary layer which further degrades the dissolution rate.

An acceptable way to reduce the mounding effect in the industry is to increase the RPMs of the paddle. By increasing to 75 RPM, the dead zone becomes smaller. Increasing to 100 RPM will reduce the dead zone further. Increasing the RPM to 250 will eliminate the dead zone all together. However, the faster the stir rate means the more stress on the tablet in the vessel. More stress than what may be seen in a stomach in the fasted state. So now the issue is that the dissolution rate may be too fast as the boundary layer is being reduced by the artifacts of the instrument.

Another option to reduce the issue of the dead zone was the incorporation of a peak vessel which can be seen in Picture 2. The peak vessel has a small mound that is centered under the paddle shaft. This acts as a blocker for the dosage unit, not allowing it to enter the dead zone. The tablet is now in the higher velocity rate of the system as shown in Figure 3. As investigated by Tahseen Mirza et al., the average percent released for high and low solubility drugs using the peak vessel increased when compared to the USP Apparatus II dissolution vessel and was comparable to the results obtained at a higher rate of paddle rotation of 75 RPM vs the original 50 RM of the method.[21]



Picture 2: Varian Peak Vessel

S.A. Qureshi developed a novel solution for the dead zone that is known as the Spindle Arm.[22] The crescent shaped spindle can be seen in Figure 4. In this instance the spindle arm would replace the paddle. Rotations were decreased as there were increased stresses applied to the dosage forms as they were pushed through the media along the bottom of the vessel. As a result, the dissolution rates were higher due to a lower boundary layer that was the result of the spindle brush arm. This manipulation of the tablet stopped further pursuit of this research.

Figure 4: Spindle Arm Research Schematic[22]

While research for improving this instrument flaw was proceeding, Bertil Abrahamsson, et. al. devised an experiment to measure the sheer forces on a tablet in a fed stomach.[23] In this experiment they rigidly suspended a tablet in a cylinder of media. The cylinder rotated allowing the media to pass by the tablet. A depiction of this experiment can be seen in Figure 5. This research was to understand the shear forces applied to the tablets in media. But its design could not be used for day to day QC testing showing the release of the drug from the tablet as the tablet had to be manipulated to be suspended in the fluid. The manipulation can cause defects of the tablet that will allow for quicker dissolution than designed.

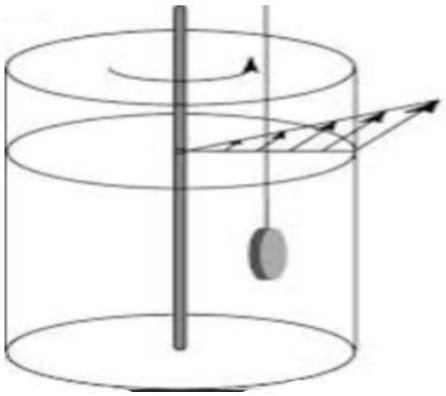


Figure 5: Illustration of Rotating beaker with Fixed Tablet[23]

This section sums up the need to look for a different mixing mechanism than the paddle due to the issues with the dead zone directly under the paddle. Also, the sheer forces on the tablets are not the same from the paddle mixing design. Somehow, the research needs to have the tablet 'bounce' around in the media, like it would in the stomach.

1.4.2 Media Volume

As discussed in 1.3, the actual volume of media in the stomach of a fasted individual who has taken one glass of water with the drug is between 260 mL and 340 mL. The dissolution test is typically run with volumes of media between 500 mL and 1000 mL, with the most common volume being 900 mL.[10]

In the stomach saliva and other excreted fluid is added to the volume while solution is passed on to the duodenum. The volume moving to the duodenum is greater than the excreted fluid and saliva being added to the contents as the stomach is working to get back to its normal 35 mL to 50 mL steady state volume. Meanwhile the duodenum also maintains its 100 mL volume.

This research will focus on actual media volumes in the fasted human model. Higher dose drugs only have up to 50 mL to play with for solubility. A 100mg dose would require a 2 mg/mL solubility level at least. What effects happen when the drug is not soluble enough and the particles drift through the duodenum undissolved? This is a question that cannot be answered by the current USP apparatus 2 system.

1.4.3 Multi Compartment Dissolution Systems

Novel new instruments were being designed to try and mimic the tablets performance in the stomach. One of the better instruments developed to simulate the human digestive system (and is being used today) is the TNO Gastro-Intestinal Model (TIM).[24] This instrument can be seen in Figure 6. It is a multi-compartment model that can simulate both the FED and FASSIF state of the human digestive model. Each compartment is designed to simulate the proper juices for digestion. Each compartment can be sampled from as needed for the experiment. And the TIM model has a patented transfer process from one compartment to the next that allows solid material to pass down the digestive track. This system can be used to evaluate food effects for the new drug entities or test that novel dosage form is releasing as it was designed to do as it hits the correct pH.

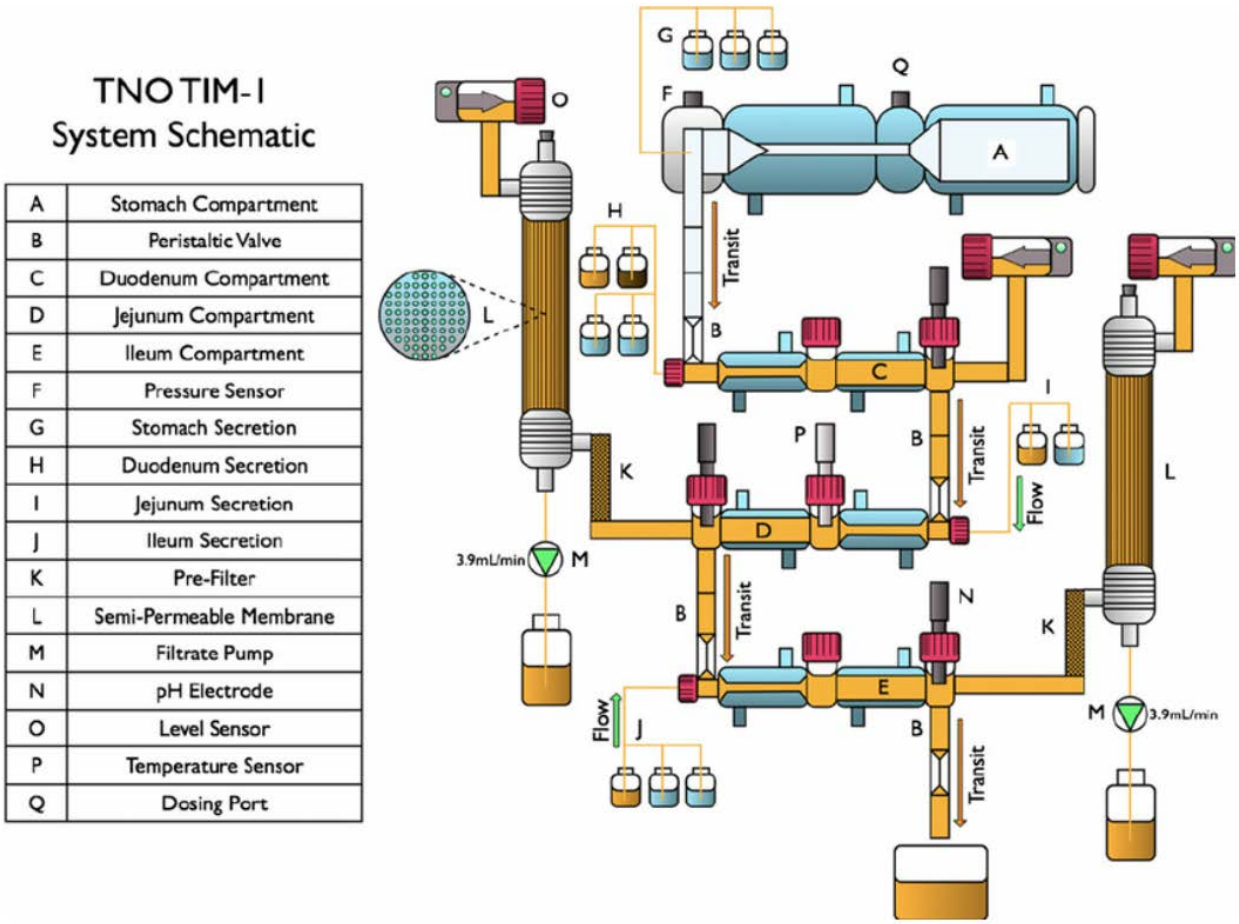


Figure 6: Schematic Diagram of TNO TIM-1 Model[25]

While the TIM-1 system is one of the most physiologically relevant dissolution apparatus for use in drug development,[5] it is not designed for day to day QC testing. The TIM-1 system is a very complicated, very large instrument. Setting up the system, performing the test, breaking down the instrument and cleaning to prepare for the next test is a full day's job that provides a single tablet result. A single USP Apparatus 2 system can be run manually in under 2 hours[26] and 2 systems can be run together with a standalone UV analysis finish and being fully documented in approximately 6.5 hours.[26] Each USP Apparatus II system runs 6 dosage units. In one day, a QC analyst can run 12 tablets with the USP Apparatus II system, or 1 tablet using the

TIM instrument. Through put is a key deliverable for a QC lab and therefore the TIM model would not meet this standard.

Deanna M. Mudie et. al. created a three-compartment dissolution system.[27] It is a clean and robust system that can be easily turned over and run again quickly. Much quicker than the TIM model. However, the problem with the three-compartment system was the mixing being utilized. The mixing is completed by a paddle. As shown before the mixing by a paddle creates the dead zone space under the paddle where the tablet will feel little to no effects of the fluid flowing over the tablet to disintegrate it. But it also has the wrong sheer force being applied to the tablet. In the stomach, fluid does not flow over the tablet. The tablet flows through the fluid. The stresses on the tablet are different as shown by Bertil Abrahamsson, et. al..[23]

Another multi compartment model developed is known as GOLEM. As discussed by Ivan Stupak et. al., the system was developed to test 4 sections of the digestive tract in line: the stomach, the duodenum, the jejunum and the ileum.[28] The system uses infusion bags that are mixed trough an 'electrically driven agitation/peristaltic system with autonomous mixing for each compartment.'[28] It is a unique system that has shown some good data. The issue with this system is focused on the robustness and repeatability. The experimental data has shown a large %RSD for the compartments. The best two that were chosen in this research had variability of 25% at the start to under 10% at the finish for the higher agitation rate and 50% at the start of the lower agitation rate to a little higher than that of the high agitation rate at the end. As a comparison, USP apparatus 2 systems goals are to be under 6 %RSD after 10 minutes.

The following goals have been developed for this research system to stand out from the current systems being developed.

1. The system will use a mixing mechanism that allows the tablet to flow through the media, rather than the media over the tablet.
2. The system will use volumes of media reflecting the fasted state. The system will not use static volume in a single chamber.
3. The system is to be reproducible. %RSD values should be tight, like the USP apparatus 2 system during method validations.
4. The system must be focused for QC. It needs to be able to be cleaned and rerun quickly. A single analysis should run the same amount of time as the current USP apparatus 2 system.
5. The system should be able to demonstrate an IVIVC relationship with the Gastro Plus modeling software.

Chapter 2. Materials and Methods

2.1 Chemicals and Standards

Table 2: Chemicals, Samples and Standards Used

Chemical	Manufacturer	Purity/Grade
Deionized (DI) Water	Amgen Lab/ Millipore Milli Q	
1 N Hydrochloric Acid (HCl)	Fluka	1.0 N
5 N HCl	JT Baker	5.0 N
Acetonitrile (ACN)	Sigma Aldrich	HPLC^{+} ($\geq$ 99.9%)
pH 4.5 Acetate Dilute Solution	Fluka	2.99 g/L sodium acetate, 1.68 g/L acetic acid
Sodium Acetate Anhydrous	Fisher Bioreagents	$\geq$ 99.0%
Glacial Acetic Acid	Sigma Aldrich	$\geq$ 99.7%
0.01 N HCl Dilute Solution	Fluka	0.01 N

2.2 Oral Solid Dosage Information

Two different oral solid dosage compounds were used for this research. One was an immediate release tablet formulation and the other was an immediate release capsule formulation.

2.2.1 Tablet Formulation

The compound used in the tablet will be referred to as Compound T. Compound T is considered a BCS class 2 drug. It was formulated into tablets of 1 mg strength using common excipients. The tablet target weight was approximately 100 mg for a 1% drug load. The pKa and solubility of the drug is shown in Table 3.

Table 3: Compound T pKa and Solubility

pKa	6.26
Solubility pH 2.4	33.9 mg/mL
Solubility pH 6.97	0.002 mg/mL

2.2.2 Capsule Formulation

The compound used in the capsule will be referred to as Compound C. Compound C is a BCS class 2 drug. It was formulated into capsules of 1 mg strength using common excipients and gelatin capsule shell. The target capsule fill weight was approximately 100 mg, for a 1% drug load as well. The pKa and the solubility of this drug is shown in Table 4.

Table 4: Compound C pKa and Solubility

pKa	5.30, 3.31
Solubility pH 1	7.7 mg/mL
Solubility pH 7	< 0.001 mg/mL

2.3 Development of Research Instrumentation

This research required the development and validation of a new instrument that met the following goals.

1. The system will use a mixing mechanism that allows the tablet to flow through the media, rather than the media over the tablet.
2. The system will use volumes of media reflecting the fasted state. The system will not use static volume in a single chamber.
3. The system is to be reproducible. %RSD values should be tight, like the USP apparatus 2 system during method validations.
4. The system must be focused for QC. It needs to be able to be cleaned and rerun quickly. A single analysis should run the same amount of time as the current USP apparatus 2 system.
5. The system should be able to demonstrate a relationship with Gastro Plus modeling where the absorbance in the modeling software would be set to zero as this instrument is focused on the dissolution of the active ingredient rather than drug absorbance.

The goals are intertwined with one another. For example, the mixing mechanism could not be developed without considering the media volumes being used first.

2.3.1 Research Instrument Concept

The research instrument was designed to be a multi compartment system. This resolves the problem of the current USP dissolution test being static. It also opens possible new insights into the passage of the active pharmaceutical ingredient through the gastric and duodenum compartments. For example, the experiment could track the dissolved active pharmaceutical ingredient as it passed from the gastric compartment through the duodenum compartment.

The research instrument used physiological media volumes present in the human stomach and the duodenum while in the fasted state. This provided a unique challenge in the development as the gastric and duodenum volumes in the fasted state are small. Wickham and colleagues recorded the gastric media in the fasted state was approximately 50 mL.[29] Mudie et al reported 35 +/- 7 mL of media was present in the fasted stomach during their research.[27] In the fasted state the duodenum media volume can be 33 mL[30] per Culen et al or 100 mL[31] per Takeuchi et al. For this research the gastric compartment volume would be set at 50 mL and the duodenum compartment volume would be 100 mL.

As medicinal directions state to take the medicine with a glass of water this experiment included the volume of one glass of water (240 mL) being introduced at the start of the experiment. The total volume of the gastric compartment at the start of the experiment is 290 mL. The system needed to expel the 240 mL of water volume to come back to the resting volumes in the gastric (50 mL) and the duodenum (100 mL) compartment by a certain time.

The time it takes the stomach to expel 50% of the 240 mL of water to reach the steady volume was reported by Mudie et al at 13 minutes.[27] By 45 minutes they reported that the steady volume of 35 +/- 7 mL is reached.[27] GastroPlus, a modeling software tool used in the pharmaceutical industry was also be used in this research. The default software value for stomach transit time is 15 minutes. This 15-minute default value was used to reach steady state volumes between the gastric and duodenum compartments in the developed apparatus. As a reminder, the steady state volumes were 50 mL for the gastric compartment and 100 mL for the duodenum compartment.

This created a problem on how the research instrument could be built where fluids were transferred in and out of each of these compartments. The flow rates needed to be adjusted during the experiment as well. The solution was to use a single line peristaltic pump with a manual control of the pump speed for each transfer. The tubing of the pump needed to have a large enough diameter to be able to move small chunks of undissolved material from one chamber to the next. This again was to simulate the possibility of what happens in the human stomach.

The volumes of the media used, and the physical presence of the transfer lines were considered when developing the mixing mechanism. The first mixing concept was to create an enclosed cylinder compartment that would slowly rotate on the axis. This would allow the tablet to continually fall through the solution. But the oral solid dosage would contact the hard side of the canister and could lead to physical manipulation of the dosage product. This meant that the dosage form would disintegrate faster in the system leading to a faster dissolution rate than what would be seen in the GastroPlus modeling. Other difficulties that arose with this concept was

how to keep the canister heated at 37℃, how to sample from the canister, and how to keep the transfer lines twisting together as the canister rotated.

The research by Abrahamsson et al discussed that the tablet jumped in the stomach from the contractions.[23] This correlated to the reciprocating nature seen in the disintegration instrument. The instrument can be seen in Picture 3. The instrument uses a reciprocating motion that moves six tablets up through media, does not break the surface of the media at the apex, and then the unit drops back down allowing the dosage unit with a weighted plastic 'lid' on top of it to be pushed back to the bottom. The system handles multiple dosage units at a time in a single vessel. The system uses more than the 50 mL minimum volume needed for this research. But the reciprocating action was promising as it mimicked the Abrahamsson concept quite well.

Picture 3: Disintegration Test Instrument

For the reciprocating mixing action design to be used a new system and a smaller sample holder had to be chosen. First, the system needed to hold multiple vessels or compartments that were kept at a steady temperature. The Agilent (Varian) disintegrator unit holds up to six vessels in a hot water bath and was chosen for the reciprocating arm and the hot water bath. The flat

bottom 900 mL glass dissolution vessel was used at each location of the system instead of the traditional 900 ml beaker vessel for the unit. Second, the USP apparatus III sample holder with a 45 µm size mesh bottom was chosen to be suspended by the reciprocating arm. The arm was set to 30 dips per minute with the total travel distance of 18 mm using a chain and stopping mechanism. This ensured the oral solid dosage form did not get lifted above the surface of the 50 mL volume in the 900 mL flat bottom dissolution vessel. The mesh screen on the bottom of the USP apparatus III holder would allow small particles of undissolved material to pass through and be sucked up in the peristaltic tubing to the next compartment.

A schematic of the research instrument is shown in *Figure 7* below. All compartments depicted in the schematic are suspended in the same hot water bath set to 37 ºC. The research instrument and the USP Apparatus III sample holder is shown in Picture 4. Due to the low media volume in each vessel, weights were added to the top to keep the dissolution vessels from popping up out of the hot water bath.

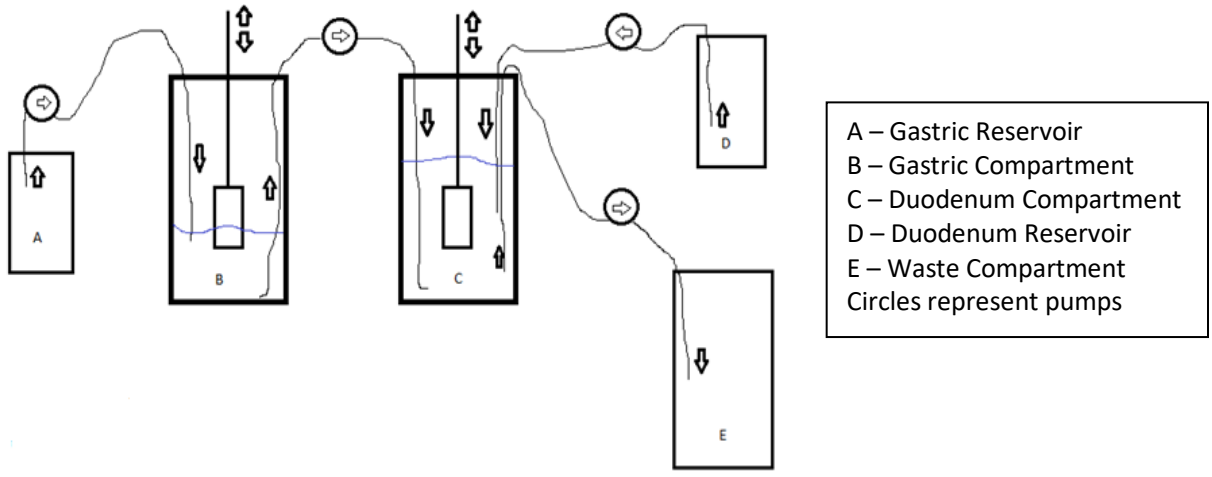


Figure 7: Design Schematic of Research Instrument

Picture 4: Research Instrument and Apparatus 3 Sample Cell

2.3.2 Research Instrument Design Refinement

Once the instrument was built the next step was determining the media to use in each compartment, the flow rates between the vessels and creating the sampling plan of the aliquots.

2.3.2.1 Media Determination

One of the goals of this research was to create an instrument that could be used in the quality control lab. To meet this goal the media needed to be easily made and have a long expiration date. While using the biorelevant media available would be a better model for the human gastric system, the media is difficult to make, and can have interference issues with standalone UV systems. The expiration date of the biorelevant media can be very short; measured in only a couple hours. The traditional dissolution media, 0.01 N HCl and pH 4.5 acetate buffer, is familiar to the quality control analysts, is easily made, and the expiration of the media can be up to a month. Therefore, this media was chosen for this research.

At the start of the experiment the gastric reservoir is filled with 200 mL of the 0.01 N HCl and the gastric compartment is filled with 50 mL 0.01 N HCl. The duodenum reservoir is filled with 200 mL of pH 4.5 acetate buffer and the duodenum compartment is filled with 100 mL pH 4.5 acetate buffer.

2.3.2.2 Determining Flowrates

There were two sets of flowrates to determine for the system. The first set of flowrates allowed the system to maintain the same volumes of 50 mL in the gastric compartment and the 100 mL in the duodenum compartment. Takeuchi et al showed the transfer rate used from the gastric reserve to the gastric compartment in the laboratory experiment was 1 mL/min.[31] In the same research the transfer rate from the gastric compartment to the duodenum compartment was also 1 mL/min to maintain a steady volume in the gastric compartment.[31] The media transfer rate from the duodenum reservoir to the duodenum compartment was 1 mL/min. Because of these two additions to the duodenum compartment (1 mL/min from the gastric compartment and 1 mL/min from the duodenum reservoir) a 2 mL/min rate was used to remove media from the duodenum compartment to the waste compartment.

The second set of flowrates were used to bring the starting volume of the gastric compartment down from 290 mL to 50 mL in 15 minutes to match the GastroPlus modeling gastric transit time parameter while maintaining the 100 mL volume in the duodenum compartment. The flowrate from the gastric compartment to the duodenum compartment was calculated to be 17 mL/min. With this amount being added to the duodenum compartment along with the 1 mL/min from the duodenum reservoir, the exit volume of the duodenum compartment was determined to be 18 mL/min to maintain 100 mL volume. A summary table of these flowrates are shown in Table 5.

Table 5: Summary of Target Transition Rates

Pump Description	Transition Flowrate (mL/min) 0 Minutes to 15 Minutes	Transition Flowrate (mL/min) 15 Minutes to End of Test
Pump 1 - Gastric Reservoir to Gastric Compartment	1.0	1.0
Pump 2 – Gastric Compartment to Duodenum Compartment	17.0	1.0
Pump 3 – Duodenum Reservoir to Duodenum Compartment	1.0	1.0
Pump 4 – Duodenum Compart to Waste Collection	18.0	2.0

2.3.2.3 Sampling

Samples were pulled from the gastric compartment and the duodenum compartment at 5, 10, 15, 20, 30, 45, and 60-minute intervals. Samples were pulled through a 45 µm filter tip filter fitted on a stainless steel canula attached to a 5 mL syringe. 5 mL's were collected into marked test tubes at each time point. 5 mL's of the relative media was then reintroduced to the appropriate compartment following each time point to maintain steady volumes. The duodenum 5 mL sample aliquots were filtered through a 5 µm filter tip filter and collected into an appropriately marked secondary test tube. All test tubes were covered with parafilm after each collection to minimize sample loss. Samples were analyzed by the appropriate analytical technique, either standalone UV or HPLC.

2.3.3 Experimental Refinement

With the initial parameters in place a series of experiments were set up to test the instrument and further refine the operating parameters. Questions were quickly answered as

well. For example, how would a capsule fare in this instrument since a capsule could float on the surface of the media in the sample cell? Would the capsule take longer to disintegrate than the traditional USP apparatus II test where the capsule is completely submerged? The research experiment showed that the part of the capsule exposed to the media did disintegrate quickly and the contents fell through the solution as it settled to the bottom of the vessel. Further, solid particles were observed being transferred to the duodenum and the waste compartments.

A few key lessons learned through the experimental refinement of the operating parameters of the instrument included optimizing the media in the duodenum compartment, improving the positions of the transfer lines, and refining the sampling timepoints.

2.3.3.1 Optimizing the Duodenum Media

The first experiment demonstrated that the media in the duodenum compartment was incorrect. The duodenum fluid is targeted be pH 4.5 acetate buffer. The emphasis was on the pH of the media. And while 100 mL of pH 4.5 acetate buffer was used to start the experiment, the1:1 mixture of the 0.01 N HCl media with pH 4.5 acetate buffer media being added to the compartment was not considered to maintain the pH. At the end of the experiment the pH was measured at 3.6 for the duodenum compartment.

Bench top experimentation was conducted to find the correct mixture to yield a final pH of 4.5. This was accomplished by mixing different pH level USP sodium acetate solutions with 0.01 N HCl on a 1:1 ratio in a hot water bath at 37 ℃. The correct 1:1 mixture contained a pH 5.0 acetate buffer mixed with 0.01 N HCl.

All subsequent experiments were corrected for this error. The starting 100 mL solution in the duodenum compartment was comprised of 50 mL of 0.01 N HCl and 50 mL of pH 5.0

acetate buffer. The duodenum reservoir was comprised of 200 mL of the pH 5.0 acetate buffer media. Standards made for the duodenum compartment analysis were dissolved in and diluted to volume with the 1:1 mixture of 0.01N HCl and the pH 5.0 acetate buffer.

2.3.3.2 Improving the Position of the Transfer Lines

The placement of the transfer lines was another important consideration discovered during the early experimentation. At first the lines were positioned so that they were all submerged. Depth of the transfer lines was not a focal point and the lines were not in a fixed position.

Results of the experiments were variable in the beginning and one main factor was the transfer lines. First, with the transfer lines loose and not fixed, more than once a line would be removed accidently during the sample pulls where the lid of the vessel or the sample cannula would move the transfer line out of the media. Second, if the lines were submerged to the bottom of the vessel, they tended to pull in the larger undissolved excipients which would block the transfer line.

Through trial and error, the best practice for the transfer lines was determined. The lines removing fluid were positioned just slightly off the bottom of the flat bottom vessel. They were taped into position. The transfer lines that delivered the volume to a vessel were fixed so the media would drip into the vessel. This allowed easier visual confirmation that the lines were not blocked by excipients during the experiment.

2.3.3.3 Improving Sample Timepoints

In the beginning of the experimentation the timepoints used were carried over from the traditional USP dissolution testing methodology. The samples were pulled at 5, 10, 15, 20, 25,

30, 45 and 60 minutes. However, in comparing the models generated from GastroPlus to the results delivered from the research instrument, it was determined that more timepoints earlier were needed. This was to better capture the max amount of drug present in the gastric compartment. The new sample timepoints were 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 45 and 60 minutes.

2.4 Calculations

Calculations were created to mathematically determine the volumes of the gastric and the duodenum compartments for the three stages of the experiment. The first stage of the experiment is where the high transfer rates expel the extra 240 mL of water present in the system. The second stage of the experiment is the transfer rates change from high to low between two sample pulls. The third stage is the transfer rates are low and set to maintain a constant volume in each compartment.

It is important to note that targeted rates are theoretical. Since the transfer rates are calibrated using peristaltic pumps with different tubing, the transfer rates in and transfer rates out for compartments will not be equal. These equations will be able to calculate the actual volume at any time of the experiment when using the calibrated transfer rates calculated prior to the experimental run.

2.4.1 Gastric Compartment Calculations

The gastric compartment starts with 240 mL DI water and 50 mL 0.01 N HCl for a total start volume of 290 mL. This volume is reduced over 15 minutes to 50 mL. The volume is targeted to remain constant for the remainder of the experiment at 50 mL. Sample time points are 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 45 and 60 minutes.

2.4.1.1 First Stage of Experiment

$$Volume_T = 290 + (TRI * T) – (HTRO * T) \qquad \text{Equation 1}$$

Equation 1 is used to determine the variable volume of the gastric compartment during the first stage of the experiment. $Volume_T$ is the volume in the gastric compartment at sample time point (T). 290 mL is the starting volume of the gastric compartment. TRI stands for the transfer rate into the gastric compartment. HTRO stands for the high transfer rate out of the gastric compartment.

An example of this equation is shown below where the volume at 10 minutes is determined with a transfer rate into the gastric compartment is 1 mL/min and the transfer rate out of the compartment is 17 mL/min.

$$290 + (1 * 10) – (17 * 10) = 130$$

2.4.1.2 Second Stage of Experiment

$$Volume_{TT} = 290 + (TRI * TA) – (HTRO * TT) – (LTRO * (TA – TT)) \qquad \text{Equation 2}$$

Equation 2 is used to determine the volume in the gastric compartment when the transfer rates change from high to low between two sample collection time points. 290 was the starting volume of the test. TA is the sample time point after the transfer rates change from high to low. TRI is the transfer rate into the gastric compartment. HTRO is the high transfer rate out of the gastric compartment. TT is the actual transition time from high transfer rate to low transfer rate. LTRO is the low transfer rate out of the gastric compartment.

An example of this equation to determine the volume at 20 minutes is shown below where the high transfer rate out is 17 mL/min, the low transfer rate out is 1 mL/min, and the transition time is 15 minutes.

$$290 + (1 * 20) – (17 * 15) – (1 * (20 – 15)) = 50$$

2.4.1.3 Third Stage of Experiment

$$Volume_T = Volume_{PT} + (TRI * (T – PT)) – (LTRO * (T – PT)) \qquad \text{Equation 3}$$

Equation 3 determines the volume in the gastric compartment when the transfer rate out of the compartment is low for the remainder of the experiment. $Volume_T$ is the volume in the gastric compartment at the current sample time point. The volume of the gastric compartment at the previous time point is denoted as $Volume_{PT}$. TRI is the transfer rate in. LTRO is the low transfer rate out. PT is the time of the previous time point.

An example of this equation to determine the volume at 30 minutes is shown below. The transfer rate in and the transfer rate out are both 1 mL/min. The volume at 25 minutes (the previous time point) is 50 mL.

$$50 + (1 * (30-25)) – (1 * (30-25)) = 50$$

2.4.2 Duodenum Compartment Calculations

The duodenum compartment starts with a volume of 100 mL. The volume is to remain steady throughout the experiment. This is made difficult as there are two lines adding media to the compartment while one removes the media. The transfer line from the gastric compartment to the duodenum compartment and the transfer line from the duodenum compartment to the waste compartment will change during the experiment.

2.4.2.1 First Stage of Experiment

$$Volume_T = 100 + (DRTRI * T) + (GCHTRI * T) – (HDCTRO * T) \quad \text{Equation 4}$$

Equation 4 is used to determine the volume in the duodenum compartment when the transfer rate from the gastric compartment and the transfer rate to the waste compartment is high. $Volume_T$ is the volume in the duodenum compartment at the current sample time point. DRTRI is the duodenum reservoir transfer rate in. GCHTRO is the gastric compartment high transfer rate in. HDCTRO is the high duodenum compartment transfer rate out of the duodenum compartment.

An example of this equation to determine the volume in the duodenum compartment at 10 minutes is shown below. At this point the transfer rate in from the gastric compartment is 17 mL/min and from the duodenum reservoir is 1 mL/min. The transfer rate out of the duodenum compartment is 18 mL/min.

$$100 + (1 * 10) + (17 * 10) – (18 * 10) = 100$$

2.4.2.2 Second Stage of Experiment

$$Volume_{TA} = 100 + (DRTRI * TA) + (GCHTRI * TT) + (GCLTRI * (TA – TT)) – (DCHTRO * TT) – (DCLTRO * (TA – TT)) \quad \text{Equation 5}$$

Equation 5 was used to determine the volume ($Volume_{TA}$) in the duodenum compartment at the sample time point after the transition rates switch from high rates to low rates. 100 mL was the starting volume in the duodenum compartment. TA is the sample time after the transition to slower rates. DRTRI is the duodenum reservoir transfer rate into the duodenum compartment. GCHTRI is the gastric compartment high transfer rate into the duodenum

compartment. GCLTRI is the gastric compartment low transfer rate into the duodenum compartment. DCHTRO is the duodenum compartment high transfer rate out of the duodenum compartment. DCLTRO is the duodenum compartment low transfer rate out of the duodenum compartment. TT is the calculated transition time for switching the transition rates from high to low.

An example of this equation to determine the volume in the duodenum compartment at 20 minutes (TA) is shown below. The transition time from high rates to low rates was 15 minutes. The high transfer rate into the duodenum compartment from the gastric compartment was 17 mL/min and from the duodenum reservoir was 1 mL/min. The high transfer rate out of the duodenum compartment was 18 mL/min. The low transfer rates into the duodenum compartment was 1 mL/min for both the gastric compartment transfer line and the duodenum reservoir line. The low transfer rate out of the duodenum compartment was 2 mL/min.

$$100 + (1 * 20) + (17 * 15) + (1 * (20\text{-}15)) – (18 * 15) – (2 * (20 – 15)) = 100$$

2.4.2.3 Third Stage of Experiment

$$\text{Volume}_T = \text{Volume}_{PT} + (\text{DRTRI} * (T – PT)) + (\text{GCLTRI} * (T - PT)) – (\text{DCLTRO} * (T – PT)) \quad \text{Equation 6}$$

Equation 6 was used to determine the volume (Volume_T) in the duodenum compartment when all transition rates were low for the remainder of the experiment. The volume of the duodenum compartment at the previous time point is Volume_{PT}. DRTRI is the duodenum reservoir transfer rate into the duodenum compartment. GCLTRI is the gastric compartment low transfer rate into the duodenum compartment. DCLTRO is the duodenum compartment low transfer rate out of the duodenum compartment. PT is the time of the previous time point.

An example to determine the volume in the duodenum compartment at 30 minutes (T) is shown below. The previous time point is 25 minutes. The two transfer rates into the duodenum compartment are both 1 mL/min. The transfer rate out of the duodenum compartment is 2 mL/min.

$$100 + (1 * (30\text{-}25)) + (1 * (30 - 25)) - (2 * (30 - 25)) = 100$$

2.5 Summary of Research Instrument Running Parameters

The following tables outline the research instruments targeted running parameters based on the instrument development studies. Each compound was qualified in each media for linearity, accuracy and sample precision (including filter studies).

Table 6: Targeted Research Instrument Parameters

Sample Holder Parameters (measured from bottom of holder)	Max Depth	Max Height	Reciprocations per Minute
	2 mm	17 mm	30
Bath Temperature	37.0 ℃ +/- 5 ℃	Time to Switch transfer rates	15 minutes
Water volume added at start of experiment to simulate glass of water			240 mL

Table 7: Targeted Sampling Time Points

Theoretical Pull Time (minutes)	Gastric Actual Pull Time	Duodenum Actual Pull Time
1	1:00	N/A
2	2:00	
3	3:00	
4	4:00	
5	5:00	5:30
10	10:00	10:30
15	15:00	15:30
20	20:00	20:30
25	25:00	25:30
30	30:00	30:30
60	60.00	60:30

Table 8: Targeted Compartment Volumes

Compartment	Initial Volume (mL)
Gastric	50
Duodenum	150

2.6 USP Instrumentation and Methodology

Both compounds T and C were tested in their validated USP apparatus II dissolution quality control methodologies to establish a baseline data set of which this research instrument is compared against. The n=6 results of this test are reported against a Q value per the USP. The Q value is a statistical result that states the test batch meets the percent amount released. There are three levels to the USP results meeting the established Q value. They are as follows. If the criteria at the first level is not met, more samples are tested and compared to acceptance criteria in the second level. If the second level acceptance criteria are not met than more samples are tested against the acceptance criteria at the third level.

1. Test six (n=6) samples of the test batch. All individual samples must meet Q + 5%. For example, if Q was set to be 75% at 45 minutes, each individual result must be at 80% or higher at 45 minutes.
2. Test a total of twelve (n=12) samples. The mean result of the twelve samples must equal the Q value and not more than 1 sample can be less than Q-15%. For example, twelve samples were tested. The mean result equaled 75% at 45 minutes. Only one result was below 60%.
3. Test a total of twenty-four (n=24) samples. The mean result of the twenty-four samples must be equal to or greater than the Q value, not more than two samples can be less than Q-15%, and not more than one sample can be less than Q-25%. For

example, at 45 minutes the average of the twenty-four samples was 75% with one sample at 59% and another sample at 49%. All other samples were higher than 60%.

The following sections outline the equipment and methodology used for Compound T and Compound C using current pharmaceutical industry standards.

2.6.1 Compound T

Compound T was validated to be tested on the USP apparatus 2 dissolution instrument followed by analysis on a UV spectrophotometer unit.

2.6.1.1 Instrumentation

The dissolution test was performed using the Distek Evolution 6300 bath in the apparatus II configuration. Samples were drawn from the vessel using Chemstation Software for multi-bath dissolution testing at set intervals and analyzed on an Agilent 8453 UV-Vis calibrated system.

Balances used during the testing were the Mettler Toledo MT5 for standard preparations and the Mettler Toledo AG 245 for the sample weights.

2.6.1.2 Methodology

Compound T was tested on a USP apparatus II dissolution bath. The media used was 0.01 N HCl. Each vessel contained 500 mL of media heated to 37.0 °C ± 0.5 °C. Temperatures were recorded at the start and end of the dissolution test. Paddle heights were measured at 25.0 to 25.5 mm from the bottom of the paddle to the bottom of the dissolution vessel. Paddle speed was set to 75 rpm. Filters used for sample collection were 10 µm full flow filters. Samples were collected at 10, 15, 30, 45 and 60 minutes. After 60 minutes the rpms were increased to 250 and an infinity pull was made at 75 minutes (15 minutes beyond the 60-minute sample pull).

The samples were analyzed using Chemstation UV Multi Bath Software. Flow cell size was 1 cm (10mm). Wavelength was 240 nm. There was no background correction. System was suitably blanked prior to analysis using the 0.01 N HCl dissolution medium.

Standards were prepared by weighing approximately 8.5 mg of the reference standard and transferring to a 250 mL volumetric flask. The standard was dissolved in and diluted to volume with the 0.01 N dissolution media and mixed well. 8.0 mL of this standard was pipetted into a 100 mL volumetric flask and diluted to mark with the dissolution media. The solution was mixed well. This was analyzed on the UV-Vis system prior to the actual run. A check standard was prepared in the same fashion. System suitability was met prior to analysis of samples.

2.6.2 Compound C

Compound C was validated to be tested on the USP apparatus II dissolution instrument followed by analysis on a HPLC instrument.

2.6.2.1 Instrumentation

The dissolution test was performed using the Distek Evolution 6300 bath in the apparatus II configuration. Samples were drawn from the vessels and analyzed on an Agilent 1100 HPLC system using Empower 3 software.

Balances used during the testing were the Mettler Toledo MT5 for standard preparations and the Mettler Toledo AG 245 for the sample weights.

2.6.2.2 Methodology

Compound C was tested on a USP apparatus II dissolution bath with the use of a Japanese basket sinker (see Figure 3) for each capsule. The media used was 0.01 N HCl. Each vessel contained 500 mL of media heated to 37.0 °C ± 0.5 °C. Temperatures were recorded at the start and end of the dissolution test. Paddle heights were measured at 25.0 to 25.5 mm from

the bottom of the basket to the bottom of the dissolution vessel. Paddle speed was set to 75 rpm. Filters used for sample collection were 10 µm full flow filters. Samples were collected at 10, 20, 30, 45 and 60 minutes. After 60 minutes the rpms were increased to 250 and an infinity pull was made at 90 minutes (30 minutes beyond the 60-minute sample pull). All samples were 1 mL aliquots.

The samples were analyzed on an Agilent 1100 HPLC system using Empower 3 software. Mobile phase was acetonitrile/DI water (30/70, v/v) with 0.1% trifluoroacetic acid. Flow rate was 1.0 mL/min. Column used was a YMC Hydrosphere C18 3 µm, 4.6 by 50 mm column. Column temperature was ambient. Injection volume was 100 µL. Wavelength detection was 325 nm. System reached a stable baseline prior to the start of the injection sequence. System suitably requirements using blanks, standards and check standards were met prior to collection of samples. Bracketing standards were implemented and confirmed to pass suitability as well for all runs.

Standards were prepared by weighing approximately 11.0 mg of the reference standard and transferring to a 250 mL volumetric flask. The standard was dissolved in 200 mL acetonitrile/DI water (20/80, v/v) with 0.1% trifluoroacetic acid (diluent) with the aid of sonication for 5 minutes. The standard was diluted to volume with diluent and mixed well. 4.0 mL of this standard was pipetted into a 50 mL volumetric flask and diluted to mark with diluent. The solution was mixed well. A check standard was prepared in the same fashion.

Chapter 3. Results and Discussion

3.1 USP Based Dissolution Results

Both Compound T and Compound C were investigational drugs being looked at to help patients. The USP based dissolution methods for both compounds were validated. Validation work included specificity, linearity, accuracy, method precision, sample precision and solution stability. Filter studies were conducted during development of the methods.

3.1.1 Compound T USP Apparatus II Dissolution Results

Compound T is an immediate release 1 mg tablet formulation. The results of the validated USP dissolution test are shown in Table 9 and Figure 8 below.

Table 9: Compound T (Tablet) USP Percent Released Dissolution Results

Vessel #	Time (Minutes)					
	10	**15**	**30**	**45**	**60**	**75**
1	90	89	92	**92**	93	92
2	91	92	94	**95**	95	94
3	93	94	94	**94**	94	94
4	93	92	92	**92**	92	92
5	94	97	95	**95**	95	94
6	95	95	95	**95**	95	95
Min	90	89	92	**92**	92	92
Max	95	97	95	**95**	95	95
Mean	**93**	**93**	**94**	**94**	**94**	**93**
Std Dev	1.7	2.7	1.4	**1.4**	1.2	1.4
%RSD	**1.9**	**2.9**	**1.5**	**1.5**	**1.3**	**1.5**

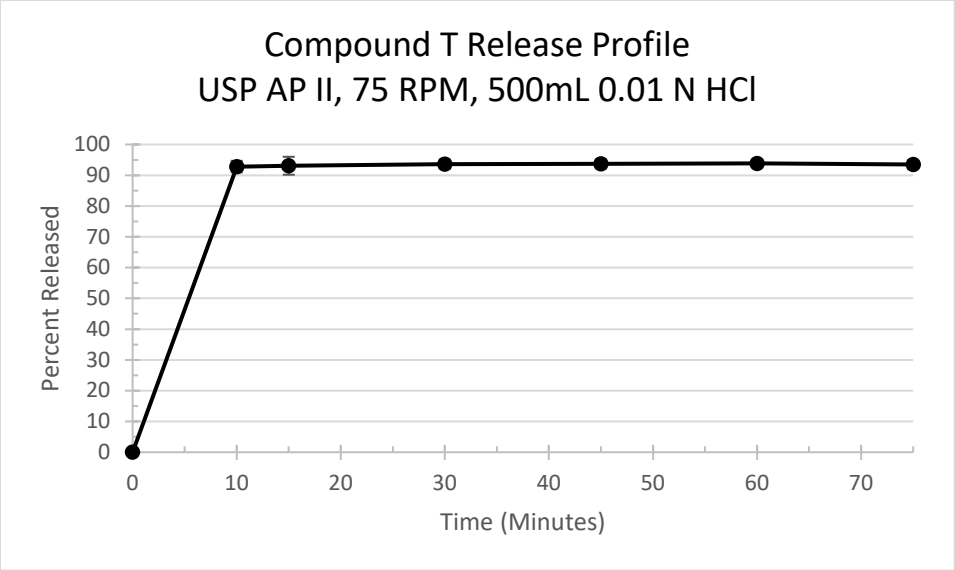


Figure 8: Compound T (Tablet) USP Release Profile

The dissolution specification set for this compound was Q = 75% at 45 minutes. The lab would conclude all results met USP L1 testing criteria where Q + 5 = 80% at 45 minutes for each individual unit. The profile would be compared to other accepted release profiles, if possible, to see batch to batch variability or previous dissolution profiles on stability to monitor any changes over time.

The data shows that by 10 minutes the active pharmaceutical ingredient (API) was fully released for this experiment. While the result did not meet 100% release, this could be explained by the low dosage of the tablet itself in the formulation. The tablet had a one percent drug load. 1mg of the active pharmaceutical ingredient mixed with 99% excipients. This high blend could lead to lower assay recoveries. Furthermore, all six dosage units have similar profiles as evident in the low percent relative standard values (%RSD) detailed in Table 9.

3.1.2 Compound C USP Apparatus II Dissolution Results

Compound C is a 1 mg gelatin capsule formulation. The results of the validated USP dissolution test are shown in Table 10 and Figure 9.

Table 10: Compound C (Capsule) USP Percent Released Dissolution Results

Vessel #	Time (Minutes)					
	10	**20**	**30**	**45**	**60**	**90**
1	95	100	98	**98**	101	98
2	90	94	95	**98**	97	98
3	95	97	96	**98**	97	96
4	98	102	99	**96**	98	100
5	95	97	101	**99**	98	97
6	95	96	99	**98**	100	97
Min	90	94	95	**96**	97	96
Max	98	102	101	**99**	101	100
Mean	**95**	**98**	**98**	**98**	**99**	**98**
Std Dev	2.4	2.8	1.9	**1.0**	1.6	1.2
%RSD	**2.6**	**2.9**	**1.9**	**1.0**	**1.7**	**1.2**

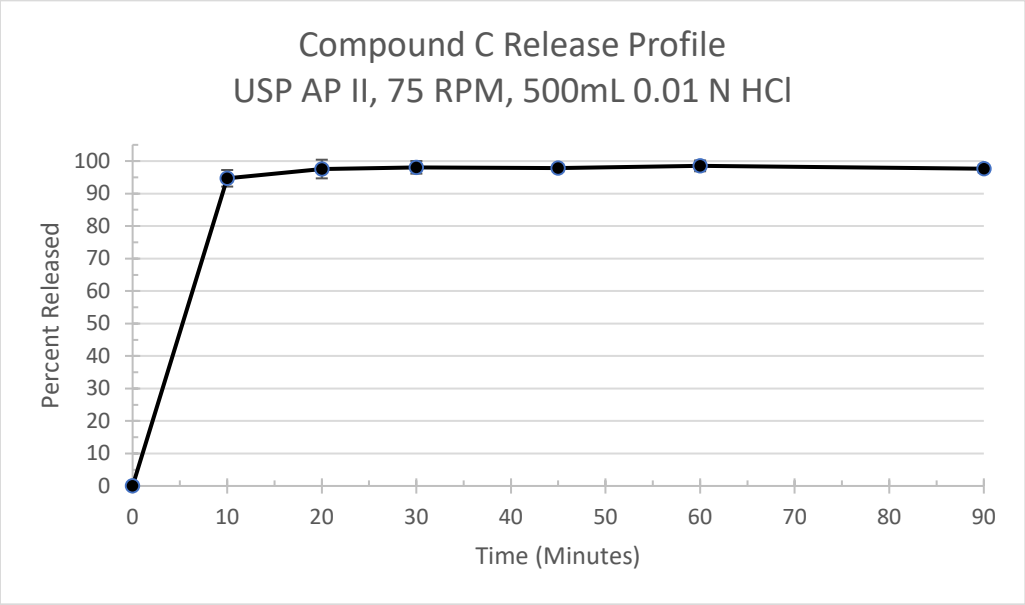


Figure 9: Compound C (Capsule) USP Release Profile

The dissolution specification for this compound was Q = 75% at 45 minutes. The lab would report the results as 'all results met USP L1 testing criteria where Q + 5 = 80% at 45 minutes for each individual unit.' The profile would be compared to other accepted release profiles, if possible, to see batch-to-batch variability or previous dissolution profiles on stability to monitor any changes over time.

The results show that by 10 minutes the API is almost completely in solution. All six dosage units have similar profiles as evident from the low %RSD values in Table 10.

3.2 GastroPlus Modeling Profiles

A GastroPlus software simulation was run for Compound T. The absorption was set to zero, the elimination was 0.25 hours, 50 mL was used for the gastric and duodenum compartment volumes, 240 mL volume was used for the glass of water and a one-minute delay was added to simulate the time for the tablet to disintegrate. Profiles of the simulated drug amount remaining in mg vs time were plotted for each compartment with data points chosen to match the instrument sampling time point. These profiles are seen in Figures 10 and 11.

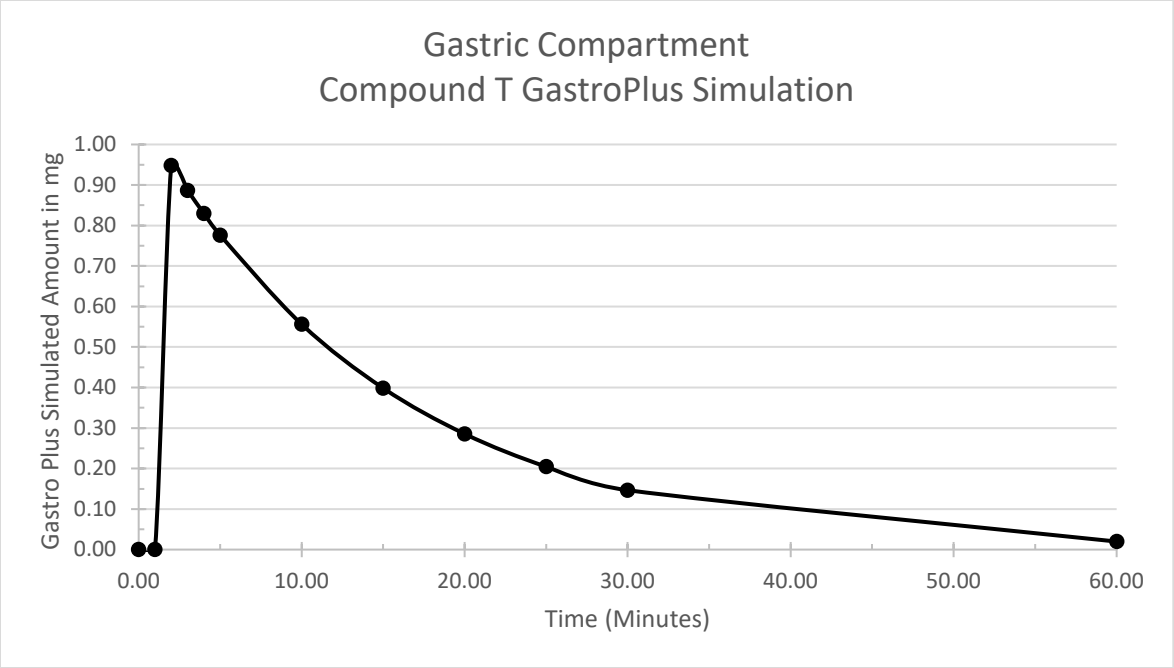


Figure 10: Compound T GastroPlus Gastric Model Simulation

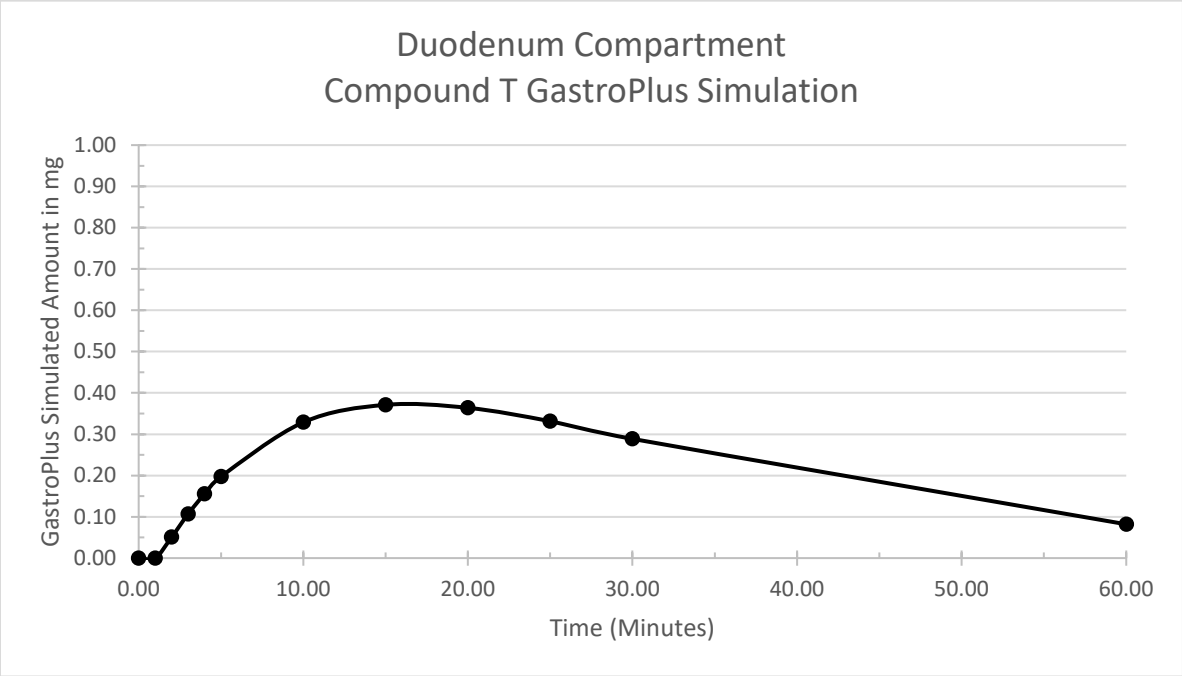


Figure 11: Compound T GastroPlus Duodenum Compartment Simulation

The gastric simulation profile shows the drug is immediately available upon the disintegration of the tablet and rapidly declines. Per the specification for the UPS Apparatus II

instrument test, at 45 minutes there is less than 10% of the drug present in the gastric compartment. At 60 minutes, less than 2% of the drug is still present in the gastric compartment as seen in Figure 10.

The duodenum simulation profile shows the amount of drug recovered increases up to almost 0.4 mg by 15 minutes and then begins to decrease steadily for the next 45 minutes. This profile shows a hold up in this compartment. Part of this reason is that the absorption was set to zero. Drug would be removed from the fluid as it is being absorbed into the bloodstream. Per the qualification the drug is fully soluble in this media at the 1mg dosage, so solubility is not a problem.

The 2-, 45- and 60-minutes time points provide the most intriguing information when compared to the original USP apparatus II test. The 2-minute time point shows that the drug is fully in solution in the gastric compartment and has already begun to move into the duodenum compartment. The USP test showed the drug was completely in solution by 10 minutes and stayed completely in solution for the rest of the test.

The 45-minute time point is where the original USP apparatus II dissolution test specification is set. The specification is Q = 75% at 45-minutes. Per the GastroPlus simulation profiles, there is less than 0.1 mg (less than 10%) of the drug present in the gastric compartment and just under 0.2 mg (20%) present in the duodenum compartment at 45-minutes.

This result highlights one of the main reasons for the need of a new dissolution instrument. By 45-minutes the GastroPlus simulation shows most of the drug left the gastric compartment as well as passed through the duodenum compartment. The USP test is unable to

depict this change over time as it is focused on the dissolution rate in a single vessel, whereas this research instrument can capture this change over time as it is a multi-compartment system.

At 60 minutes the drug present is present in both the gastric and duodenum simulations. The gastric simulation has less than 2% remaining, where the duodenum has about 8% remaining. This falls in line with the fact that 100% of solution is never fully emptied from the gastric compartment. A fraction of the solution is being constantly transferred (about 2% per minute) to the duodenum following second order kinetics.

3.3 Compound T Research Instrument Results

3.3.1 Compound T 100 mL Duodenum Volume

Compound T was optimized on the research instrument per the parameters listed in Chapter 2. The differences were that the duodenum compartment had 100 mL starting volume, the flow rate leaving the duodenum compartment was less than the targeted 2.0 mL/min transfer rate which allowed for a hold up effect in the duodenum with and ending volume greater than 100 mL, and the first sample was pulled at 5 minutes. As a result, the highest spike of the drug in solution was not captured. Results of the experiment are show in Figures 12 and 13.

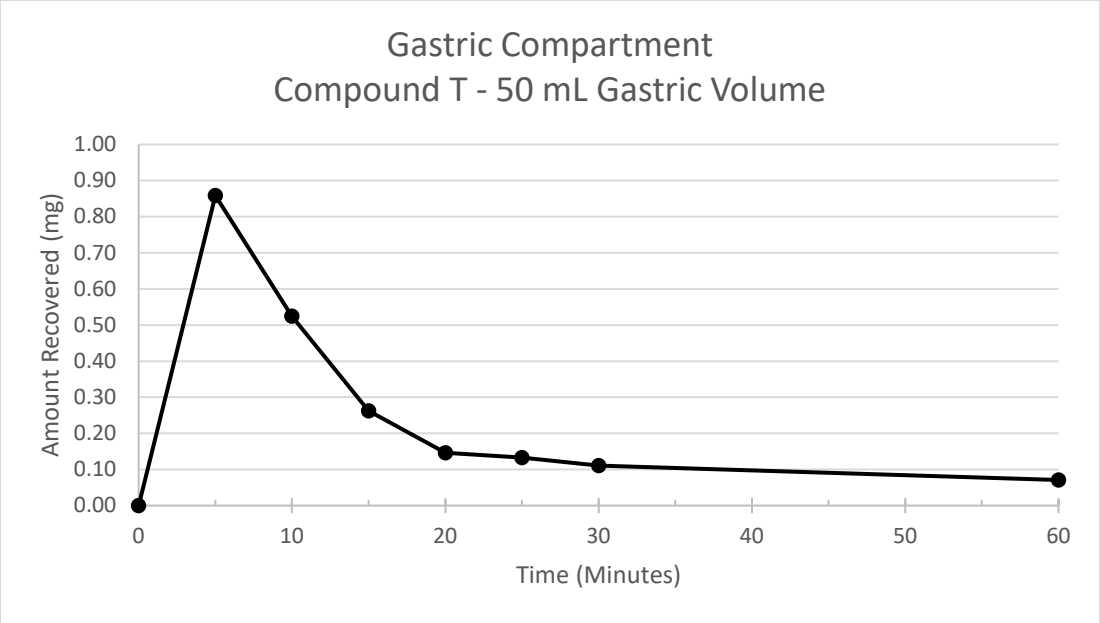


Figure 12: Gastric Compartment Results for Compound T – 100 mL Duodenum Volume

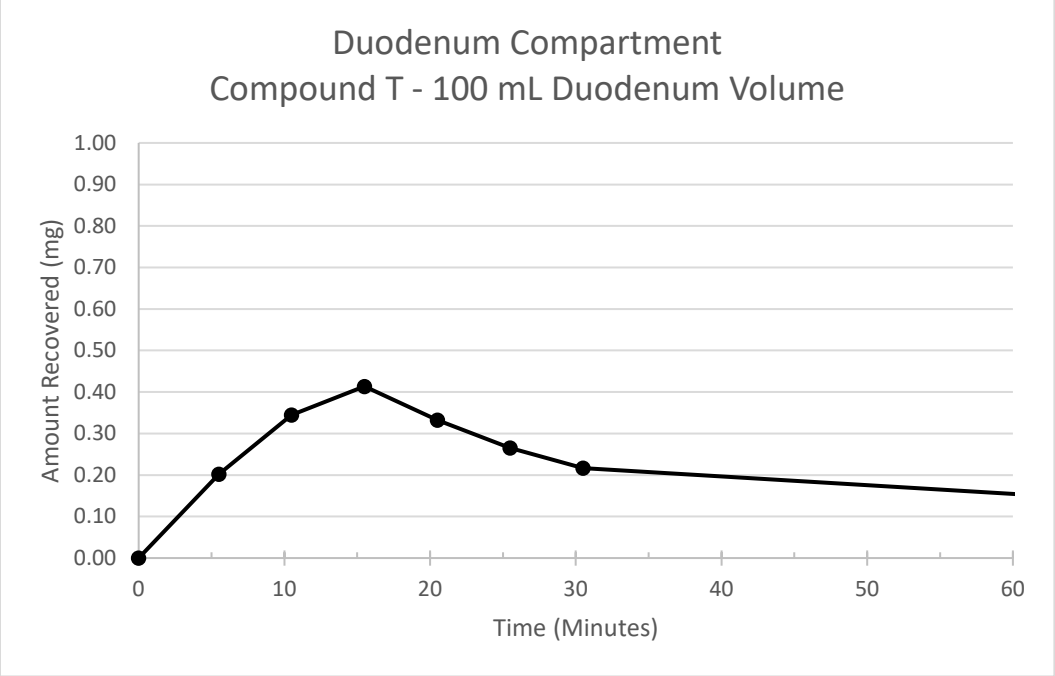


Figure 13: Duodenum Compartment Results for Compound T – 100 mL Duodenum Volume

The tablet was observed to fully disintegrate by 5 minutes. The tablet formed a mound of excipients after the tablet disintegrated as per previous experiments. The mound of excipients was directly below the reciprocating cylinder. As the experiment progressed the excipient

mound expanded into rings of powder around the center of the flat bottom vessel. This result demonstrates the instruments ability to negate the phenomena known as mounding or coning in the dissolution apparatus 2 bath through the reciprocation mechanism of mixing.

The tablet disintegration observation was backed by the data seen in Figure 12. The plot had the highest mg recovered at 5 minutes. After, there was a rapid decline of the amount of drug recovered during the high flow rate stage of the test. When the system switched over to the slower flow rates the loss of the drug in the gastric compartment flattened out. There was still 7% of the drug present in the gastric compartment at 60 minutes.

The duodenum compartment result showed that the highest mg recovered of Compound T was at the 15 minutes and 30 seconds pull with 41% mg recovered. This was significantly different to previous experimental runs. Switching the flow rate out of the duodenum compartment to be lower than what was coming into the compartment created a hold up of the material in the duodenum compartment. The duodenum compartment also showed that the drug was still present at 60 minutes at a level double of what was seen in the gastric compartment.

It is important to recall that this experiment does not factor in absorption. This is a QC test to see how fast the formulation releases the drug and how fast the drug moves to the duodenum compartment. With absorption included the amount recovered in the duodenum would be lower as the drug would be absorbed in the duodenum in addition to the transition to the next compartment. This experiment shows that the greatest amount of the drug is available to be absorbed at about 15 minutes.

3.3.2 Compound T 50 mL Duodenum Volume

The research instrument was run per the parameters in chapter 2. The single difference in this experiment was the volume in the duodenum compartment was set to 50 mL. This was to match the volume used in GastroPlus. Further the results were compared directly to the GastroPlus simulation model in section 3.2.

Using the calculations discussed in chapter 2.4, the amount of drug recovered for each compartment at each time point was calculated and populated in Table 11. The final volume in each compartment was used to ensure the volumes were accurate for each sample calculation.

Table 11: Drug Recovery Results for Compound T – 50 mL Duodenum Volume

Gastric Compartment		Duodenum Compartment	
Time (Minutes)	mg Recovered	Time (Minutes)	mg Recovered
1	0.7669	N/A	
2	0.7854		
3	0.7822		
4	0.7814		
5	0.7001	5.5	0.1372
10	0.3936	10.5	0.1399
15	0.1460	15.5	0.1245
20	0.1128	20.5	0.0979
25	0.1033	25.5	0.0780
30	0.0839	30.5	0.0674
60	0.0452	60.5	0.0411

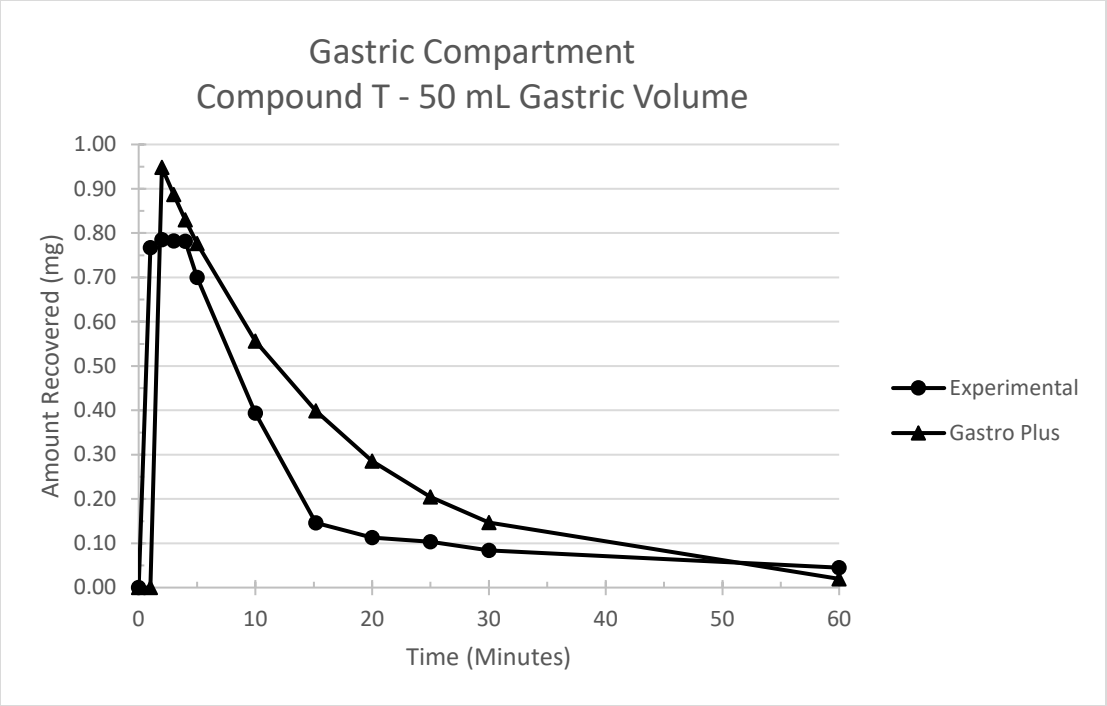


Figure 14: Gastric Compartment Results for Compound T – 50 mL Duodenum Volume

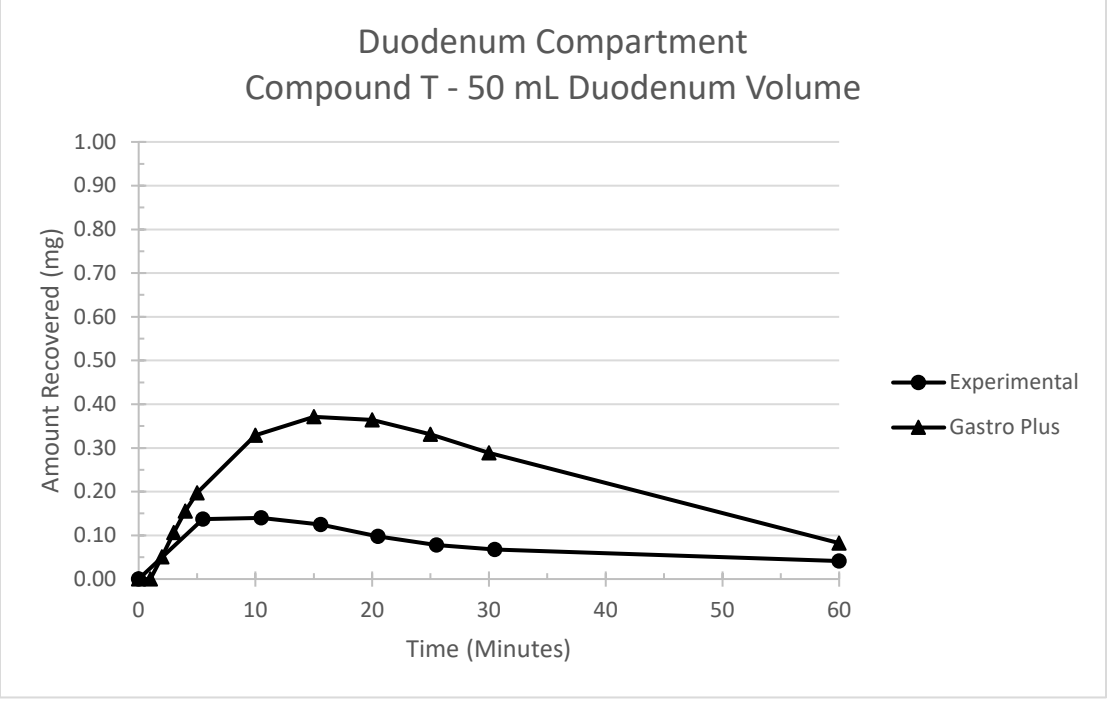


Figure 15: Duodenum Compartment Results for Compound T – 50 mL Duodenum Volume

The gastric data from the research instrument showed the amount of drug recovered in the first five minutes had plateaued at approximately 80%. The experimental data did not reach

the same spike level as the GastroPlus simulation had predicted. This was because the tablet does not instantaneously disintegrate releasing all drug at once. The drug is released as the tablet disintegrates and as the first amount of the drug is in solution it is moved quickly to the duodenum compartment. It goes to reason the same occurrence would happen in the human digestive tract as well. This demonstrates how this instrument is different from GastroPlus in that the instrument can show the importance in the formulation disintegration as it relates to the drug being in solution.

The duodenum compartment data did not have a similar profile to the GastroPlus simulation. The highest the experimental data was able to recover was about 14% at 10.5 minutes. One reason for this difference was the fact that even though zero absorption was used for the drug, the GastroPlus simulation accounted for absorption of the fluid. After discussing this internally, there was a decision to increase the volume of the duodenum compartment to offset this absorption factor embedded in the software.

3.3.3 Compound T 150 mL Duodenum Volume

This experiment followed the parameters as outlined in chapter two with the initial volume of the duodenum compartment at 150 mL. The tablet was observed to fully disintegrate by 2 minutes.

The amount of drug recovered for each compartment at each time point was calculated and populated in Table 12. The final volume was used to ensure the volumes were accurate for each sample calculation. The plots of the two compartments compared to the GastroPlus simulation are seen in Figures 16 and 17.

Table 12: Drug Recovery Results for Compound T – 150 mL Duodenum Volume

Gastric Compartment		Duodenum Compartment	
Time (Minutes)	mg Recovered	Time (Minutes)	mg Recovered
2	0.8796	N/A	
3.17	0.7311		
4	0.6685		
5	0.5827		
6	0.5293	6.5	0.3250
11	0.2892	11.5	0.3121
16	0.1143	16.5	0.3014
21	0.0876	21.5	0.2784
26	0.0680	26.5	0.2462
31	0.0512	31.5	0.2288
61	0.0193	61	0.1703

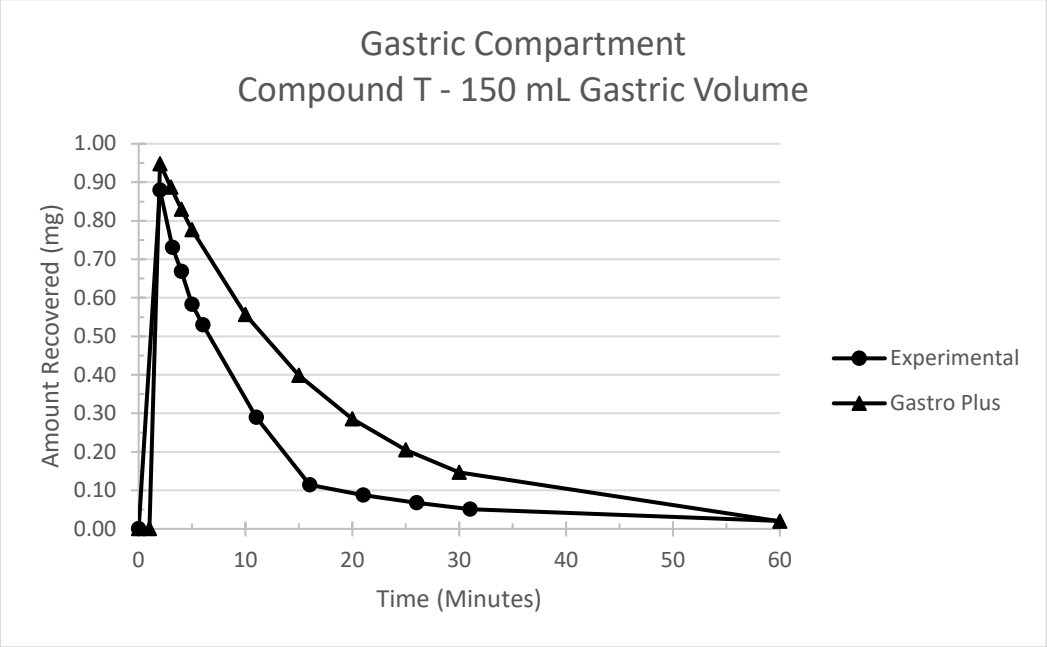


Figure 16: Gastric Compartment Results for Compound T –150 mL Duodenum Volume

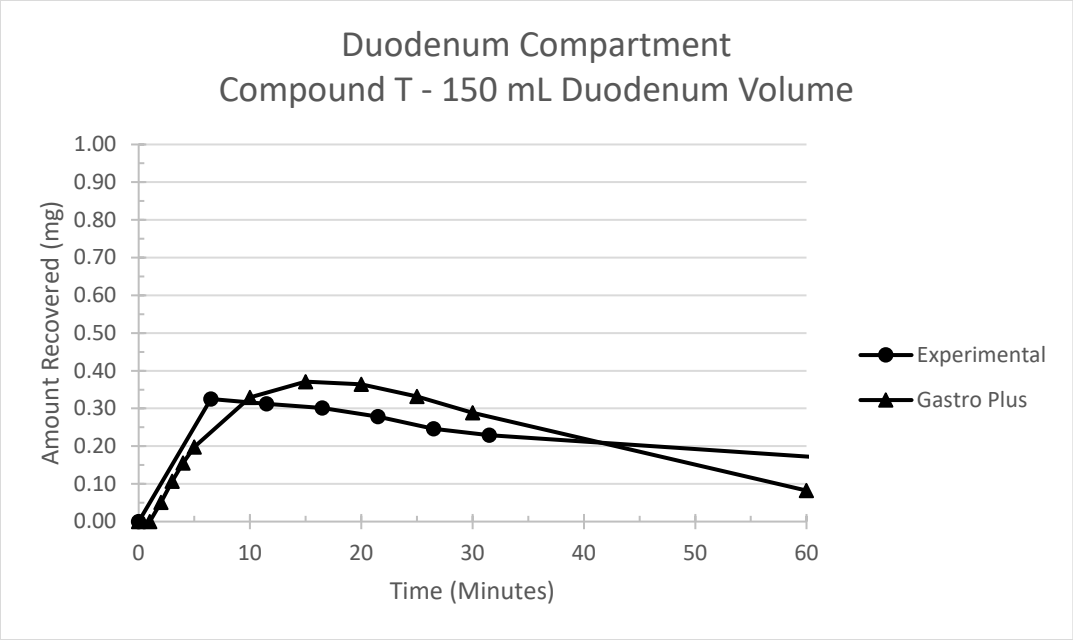


Figure 17: Duodenum Compartment Results for Compound T – 150 mL Duodenum Volume

The data from the research instrument showed similar profiles in the gastric compartment and the duodenum compartment when compared to the GastroPlus model. The use of the 150 mL starting volume in the duodenum compartment had held up the amount of drug in solution in the duodenum compartment. In previous experiments, it was difficult to get above 20% as the drug was moving out of the compartment so quickly with the lower starting volumes.

The research instrument parameters used for this experiment were repeated two more times for a total of n=3 reproducibility trial of the instrument and discussed in section 3.3.4.

3.3.4 Compound T – 150 mL Duodenum Volume, Reproducibility

Reproducibility of the research instrument was tested by running two more experiments, for a total of 3 experiment runs, using the parameters in Chapter 2. The experimental runs were completed on different days.

The amount of drug recovered for each compartment at each time point was calculated utilizing the equations in chapter 2.4 and populated in Tables 13 and 14. The final volume was used in all experiments to ensure the volumes were accurate for each sample calculation. The average plots of all three 150 mL duodenum starting volume experiments were compared to the GastroPlus simulation as seen in Figures 18 and 19.

Table 13: Compound T –150 mL Duodenum Volume, Run 2

Gastric Compartment		Duodenum Compartment	
Time (Minutes)	mg Recovered	Time (Minutes)	mg Recovered
1	0.8950	N/A	
2	0.7927		
3	0.7360		
4	0.6598		
5.00	0.5939	5.50	0.2285
10.00	0.3836	10.50	0.1889
15.17	0.1556	15.50	0.2025
20.00	0.1156	20.50	0.1989
25.00	0.0839	25.50	0.1671
30.00	0.0567	30.50	0.1430
60.00	0.0090	60.50	0.1142

Table 14: Compound T –150 mL Duodenum Volume, Run 3

Gastric Compartment		Duodenum Compartment	
Time (Minutes)	mg Recovered	Time (Minutes)	mg Recovered
1	0.7471	N/A	
2	0.7791		
3	0.7508		
4	0.6737		
5.00	0.6356	5.50	0.0711
10.00	0.4169	10.50	0.1396
15.17	0.1674	15.50	0.1846
20.00	0.1250	20.50	0.1683
25.00	0.0881	25.50	0.1551
30.00	0.0613	30.50	0.1387
60.00	0.0106	60.50	0.1166

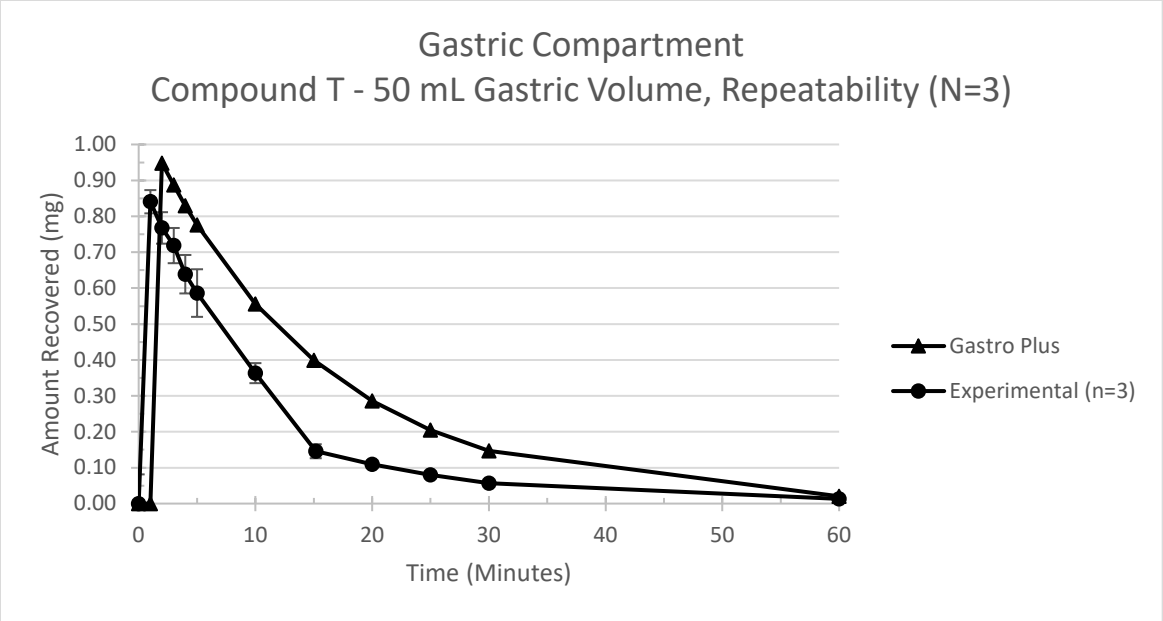


Figure 18: Gastric Compartment Results for Compound T –150 mL Duodenum Volume, Reproducibility (N=3)

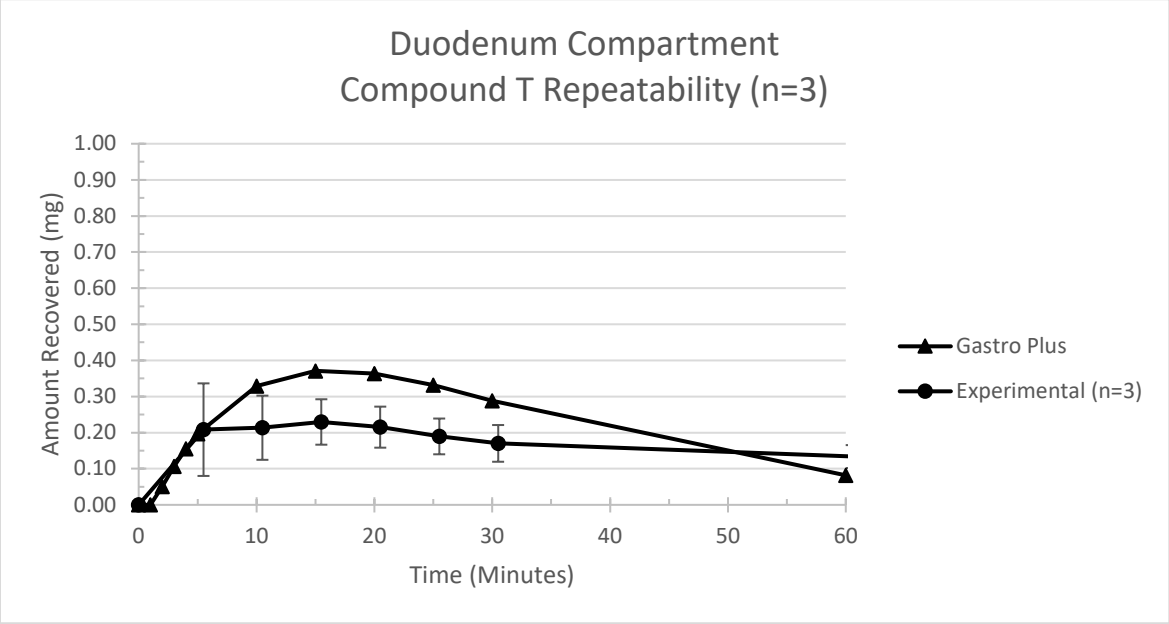


Figure 19: Duodenum Compartment Results for Compound T –150 mL Duodenum Volume, Reproducibility (N=3)

Figure 18 depicts the tight results in the research instrument for the gastric compartment.

The average plot in the gastric compartment showed a similar curve to what was predicted from

the GastroPlus simulation. The research instrument plot demonstrated a faster evacuation of the drug from the compartment than GastroPlus prediction model.

The duodenum compartment results were not as tight as the gastric compartment results. Both plots have error bars signifying one standard deviation. Experiment one was completed with the instrument being dismantled after its completion. The research instrument was reassembled and kept in the same configuration for experiments two and three. Experiments two and three were run on different days.

The first experiment showed a good comparison to the GastroPlus simulation. The second and the third experiments showed lower amount recoveries in the duodenum compartment. The final volumes showed there were greater hold ups in experiment two and three compared to experiment one. This should have had experimental runs two and three see higher amounts recovered as seen in the experiment with 100 mL duodenum volume. This was due to the theory that more solution with drug was entering the duodenum compartment than solution with drug leaving the compartment. But this was not the case. The analytical data showed material moved quickly through the second compartment into the waste compartment.

However, the results did show a good correlation to the overall GastroPlus prediction model and therefore these parameters as outlined in Chapter 2 were used for Compound C to see the correlation to the GastroPlus model.

3.4 Comparison of Compound C to GastroPlus

Compound C experiment run was conducted using the parameters outlined in Chapter 2. The end volumes were used for all calculations to ensure accurate volumes were used for the equations.

The capsule was observed to float on top of the gastric media. The capsule ruptured at approximately 1 minute and 30 seconds into the experimental run. The capsule remained floating while powder slowly escaped through the rupture on the bottom of the capsule. It was observed that powder continued to fall out of the capsule over the course of the next 5 minutes. The pumps were observed to have functioned properly throughout the run without any visible line blockage from the particles.

Using the calculations discussed in chapter 2.4, the amount of drug recovered for each compartment at each time point was calculated and populated in Table 15. The plots of the two compartments were overlaid with the GastroPlus simulation prediction for Compound C and are seen in Figures 20 and 21.

Table 15: Drug Recovery Results for Compound C

Gastric Compartment		Duodenum Compartment	
Time (Minutes)	mg Recovered	Time (Minutes)	mg Recovered
1	0.0623	N/A	
2	0.0496		
3	0.1685		
4	0.3634		
5.00	0.4686	5.50	0.0590
10.00	0.3970	10.50	0.3058
15.17	0.1413	15.50	0.5133
20.00	0.1143	20.50	0.3781
25.00	0.0858	25.50	0.3372
30.00	0.0685	30.50	0.3029
60.00	0.0204	60.50	-0.0233

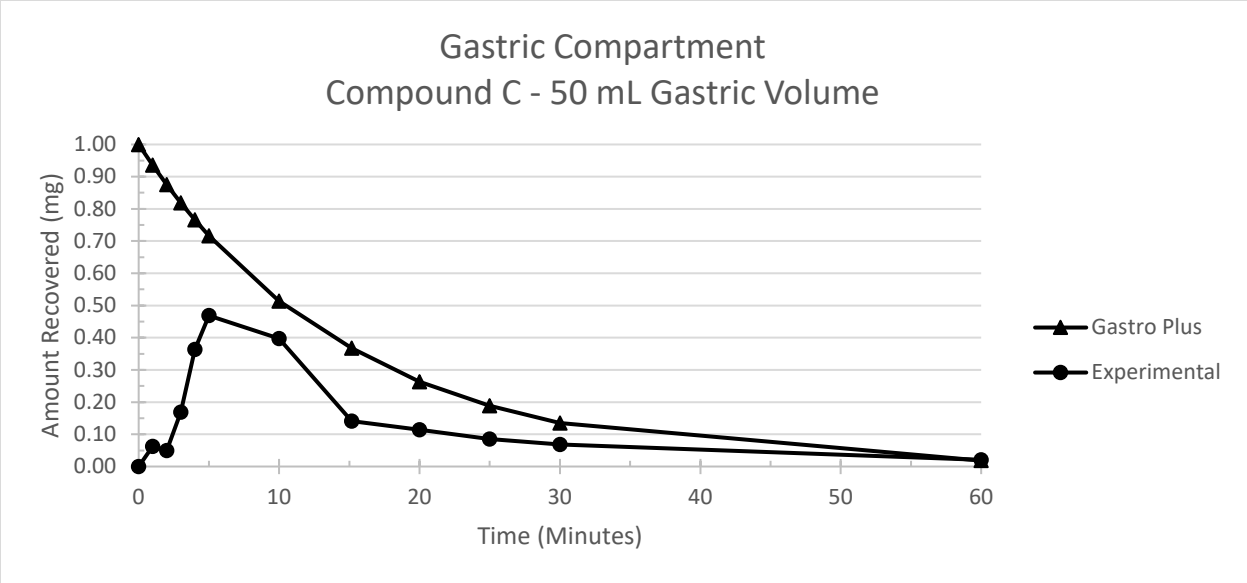


Figure 20: Gastric Compartment Results for Comparison of Compound C GastroPlus

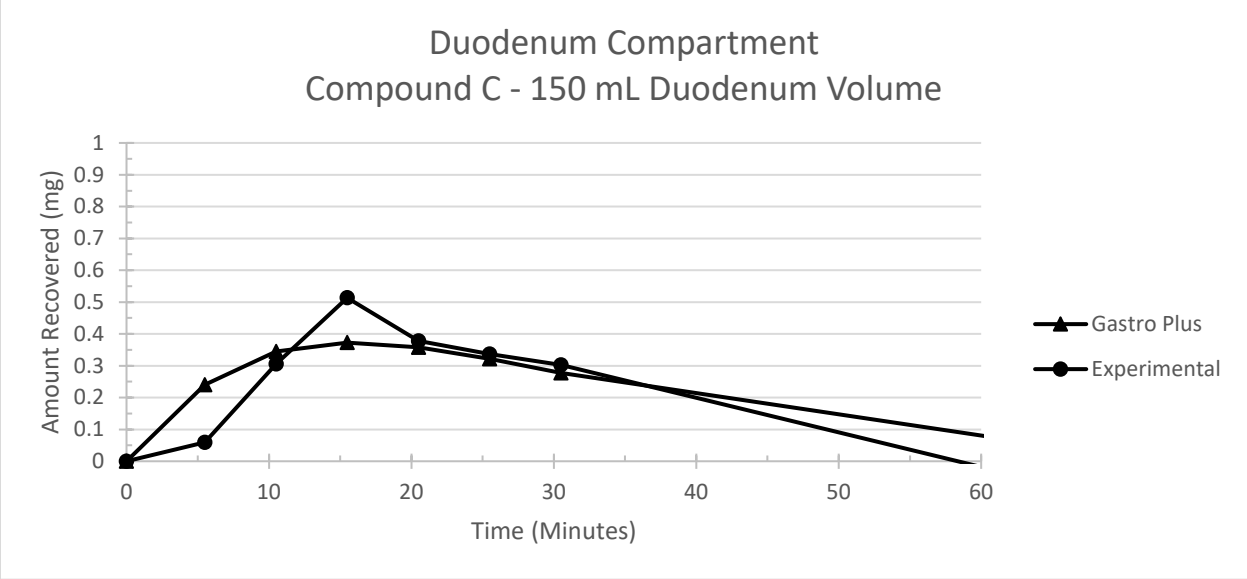


Figure 21: Duodenum Compartment Results for Comparison of Compound C GastroPlus

The research instrument plot for Compound C in the gastric compartment was significantly different than the GastroPlus simulation data. The reason was that the GastroPlus simulation assumed 100% of the drug was available in the gastric compartment at the start of the experiment for an IR capsule. This was not the case as shown with the research instrument results. The observation that the powder slowly fell from the rupture into the gastric

compartment over 5 minutes from the time that capsule first ruptured helped to validate the analytical results. The drug was slowly released from the capsule over time. The GastroPlus simulation failed to consider this slow release of the drug over time through this rupture.

The research instrument data for the gastric compartment showed a slight dip from the first and second pull. This was possibly caused by a delay in the full rupture of the capsule. Some of the drug was released before the excipients plugged up the rupture hole in the capsule shell. Then the capsule disintegrated further allowing more material to be released over time.

The research instrument duodenum compartment results matched well with the GastroPlus simulation. The slower uptake of amount recovered correlates to the slower release of the drug into the gastric compartment. It was noted at the end of the experiment there was a negative analytical value obtained for the sixty-minute timepoint due to the subtraction of the placebo absorbance. These results were based off the assumption of 100% capsule absorbance interference at each time point. Further work would be needed to investigate the actual placebo absorbance interference throughout the run as the interference would degrade over time as the placebo material is passed onto the waste compartment.

The data from Compound C did not match with the GastroPlus simulation when using the parameters established with Compound T for the gastric compartment. However, the differences noted in the gastric compartment help to illustrate why this research instrument could improve the predictive capability of GastroPlus. The fact that GastroPlus simulation did not consider the actual release of the material into the gastric compartment over time led to an error of material being available in the duodenum compartment faster than observed.

In the end, the parameters in Chapter 2 are correct to be used to match with GastroPlus simulations. The observations made during the actual experimental run related to the disintegration of the formulation over time plays the biggest role in seeing the differences between the GastroPlus simulation model and the results from the research instrument.

As discussed in Chapter 1, the dissolution test is important as it is the only analytical test used that monitors the disintegration of the oral solid dosage and the dissolution of the active pharmaceutical ingredient in media. This research instrument is proven to be able to measure both the disintegration of the oral solid dosage as well as the amount of drug released or available in both the gastric compartment and the duodenum compartment over time.

3.5 Compound T Experiment with Higher Gastric pH and Sip of Water

The research instruments running parameters from Chapter 2 were modified for this experiment to see the impact of the profiles for the gastric compartment and the duodenum compartment with the following differences to simulate an individual that had a higher stomach pH and used only a sip of water rather to take the medicine rather than a full glass. The parameter differences were:

- Gastric fluid and the fluid to be mixed with pH 5.0 Acetate buffer was 0.0001 N HCl. The pH of this media is 4.0.
- The amount of DI water added at the start of the experiment was 15 mL rather than the 240 mL for a full glass of water.
- Due to the low volume of the water added, it was assumed that the gastric emptying event would not occur in the stomach. Therefore, all transfer rates were kept at the lower steady state rates for the full length of the experiment.

Equations 3 and 6 in chapter 2.4 were used to calculate the volumes for each time point of this experiment because only the low transfer rates were used for the entire experiment. The amount of drug recovered for each compartment at each time pulled was calculated and populated in Table 16. The plots of the two compartments are seen in Figures 22 and 23.

Table 16: Drug Recovery Results for Compound T, Higher Gastric pH and Sip Volume of Water

Gastric Compartment		Duodenum Compartment	
Time (Minutes)	mg Recovered	Time (Minutes)	mg Recovered
1:00	0.4225	N/A	
2:00	0.6219		
3:00	0.6152		
4:16	0.5900		
5:00	0.5759	5:30	0.0275
10:00	0.5429	10:30	0.0451
15:00	0.4684	15:30	0.0560
20:00	0.3826	20:30	0.0661
25:00	0.3076	25:30	0.0837
30:00	0.2448	30:30	0.0982
60.00	0.0919	60:00	0.1215

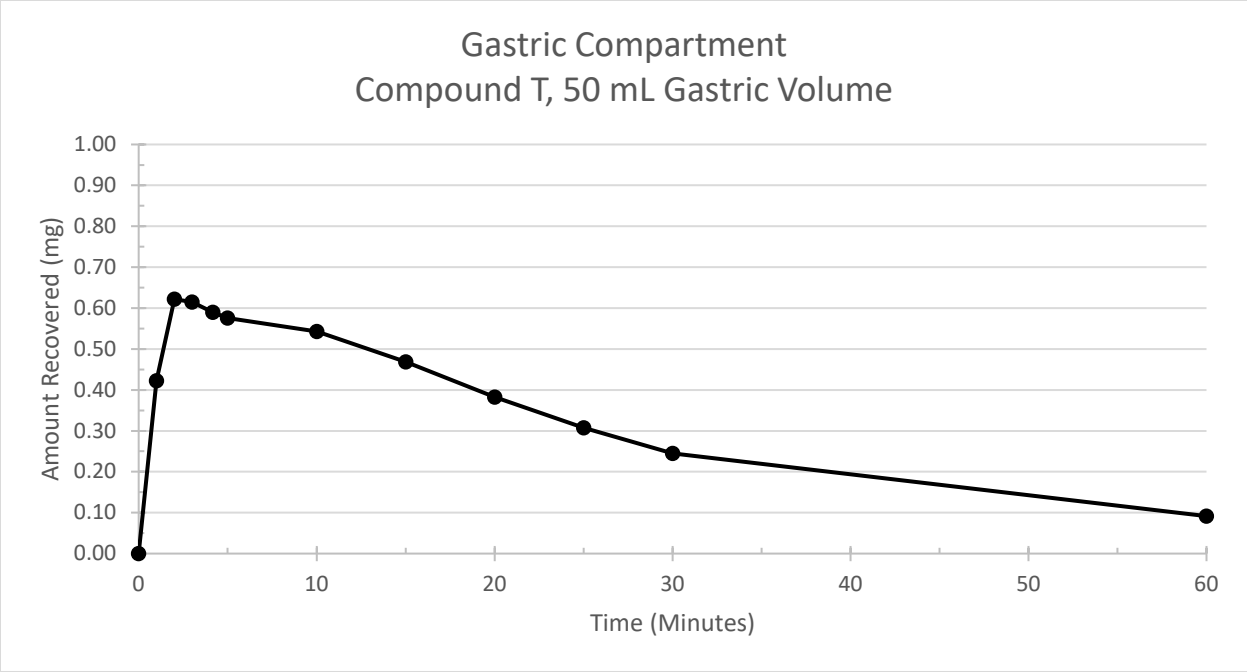


Figure 22: Gastric Compartment Results for Compound T, Higher Gastric pH and Sip Volume of Water

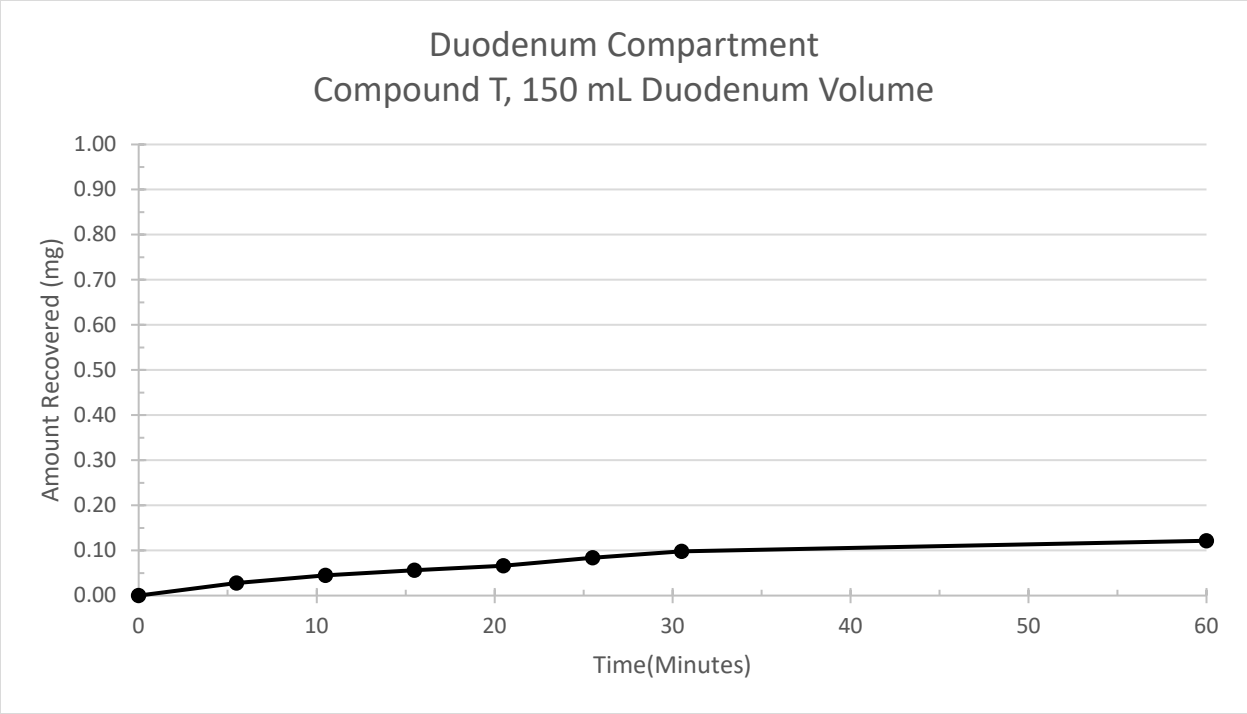


Figure 23: Duodenum Compartment Results for Compound T, Higher Gastric pH and Sip Volume of Water

The data captured for this experiment assumed that the gastric emptying event would not occur when a sip of water was taken with the medication. The volume in the stomach would not be great enough to have the event occur. Therefore, transfer rates from each compartment were kept at the lower flow rates for the whole experiment.

With that assumption, the data depicts the importance of taking the medication with a full glass of water. The drug was held up in the stomach longer than seen in previous experiments that had the gastric emptying event. The drug did not have a large spike of the drug in the gastric compartment. This could have been due to the higher pH being unable to disintegrate the tablet as quickly to deliver the large bolus of the drug up front as seen in the 0.01 N HCl media.

The duodenum compartment data showed a very low amount of the drug present throughout the length of the experiment. This is logical as each minute that passed only 1 mL of

the gastric media was transferred to the duodenum compartment. That related to approximately 1.5% of the drug present in the gastric compartment being transferred to the duodenum compartment every minute. In contrast, when the higher transfer rates are being applied the percent of the gastric media being transferred to the duodenum compartment starts at almost 6% and ends at just over 25%. The larger volume being transferred relates to larger amount of the drug being transferred in the first 15 minutes with the gastric emptying event. The consumption of the full glass of water to trigger the gastric emptying event is critical to move the drug to the duodenum compartment quickly.

The conclusion of this experiment is that a patient with a high gastric pH that took the medication with a sip of water would possibly result in the patient not feeling the full effect of the medication as it would not hit the efficacious dose in the body or the patient would have a longer delay in feeling the effects of the medication compared to a healthy individual who took the medication with a full glass of water and would have most of the drug in the duodenum compartment by 15 minutes based off of the Gastro Plus simulation and the experimental results displayed above.

Chapter 4. Conclusion and Next Steps

4.1 Conclusion

A two-compartment dissolution research instrument with a reciprocating mixing technique was developed and validated for use with two compounds, Compound C and Compound T. The instrument was designed to use bio relevant volumes and flow rates to mimic dosing in the fasted state. The reciprocating mixing proved to be effective to eliminate effects of coning. The peristaltic pumps demonstrated the ability to move solid particles from compartment to compartment, allowing undissolved drug to potentially pass through the system.

The operating parameters for the two-compartment research instrument were modified to match GastroPlus simulations using Compound T. These operating parameters were repeated using Compound T to demonstrate reproducibility of the results. The operating parameters used for Compound T were applied to running Compound C on the research instrument to compare to the GastroPlus simulation prediction. There were differences in the research instrument profiles for Compound C versus the GastroPlus simulation prediction. This was explained due to a delay in the release of the drug after the capsule ruptured which was not accounted for in the GastroPlus simulation.

The two-compartment research instrument was further tested by modifying the pH of the gastric compartment and adjusting the amount of water taken with the Compound T. The reason for this experiment was to mimic the real-world situation of patients who have a higher gastric pH or patients who take acid reducing agents. The assumption was that with a sip of water the gastric emptying event would not occur, and the low flow rates were used for the length of the experiment. The resulting profiles were significantly different compared to the original results. This was a result of using the slower flow rates and the observations made that the tablet

disintegrated slower, possibly due to the higher pH of the gastric media. This was an easy manipulation of the instrument that provided data showing the importance of taking a full glass of water with Compound T as well as the potential effects of the higher gastric pH on disintegrating the tablet, solubility and dissolution of the active pharmaceutical ingredient.

4.2 Next Steps

The instrument could be tested with higher dose drugs with low solubility. This would allow the ability to see how much undissolved drug moves through the gastric and duodenum compartments. It could also be possible to add on an additional compartment to simulate the intestinal fluids which would have much longer time points incorporated with that compartment.

The instrument could be expanded for research purposes, rather than quality control testing, by exploring the FED state. The fluids would need to include the appropriate fats and enzymes as seen in bio relevant media to simulate the gastric juices when food is present in the stomach.[14-18] The enzymes could assist in disintegration of the oral solid dosage units. For example, enzymes can play a role in preventing a known issue of crosslinking seen in the standard USP media for dissolution testing of gelatin capsules.[10-11]

In the FED state the research instrument would need to have a food surrogate present in the mixing chamber. The food would impart different forces, crushing and grinding, on the oral solid dosage unit than seen with this current version in the fasted state. This would be added forces applied to the dosage form that could be accounted for with further development of the mixing mechanism with semi soft beads added to the Gastric compartment to simulate the solid undigested food.

With the food surrogate present, the mixing mechanism of this research instrument would need to be revisited. The sample holder would not allow the proper interaction of the food surrogate with oral dosage unit. An instrument where a bag containing the food surrogate, biorelevant media, and the solid oral dosage unit that can be compressed at different angles could be one option to explore.

Additional work could also be focused focus on adding absorption considerations for each compartment of the research instrument for research and development purposes. One possible consideration for this experiment would be utilizing the Caco-2 continuous dissolution/Caco-2 system by Mark J. Ginski et al..[32] The research had a pump from the dissolution vessel to the Caco-2 chamber as shown in Figure 8 below. A similar concept could be used for this research instrument there by keeping the same mixing mechanism of the reciprocating cylinder as well as keeping the multi-compartment system.

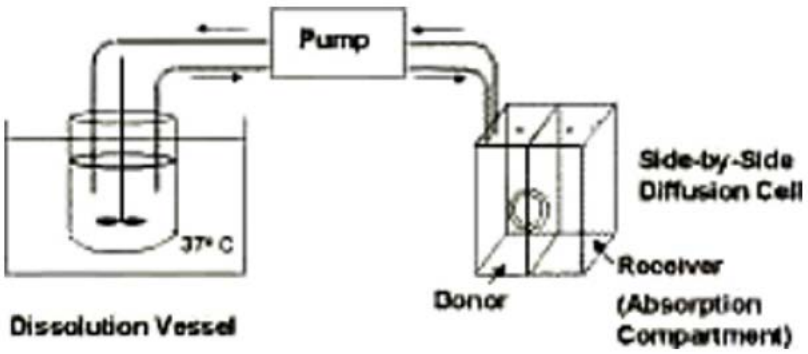


Figure 24: Schematic of the continuous dissolution/Caco-2 system[32]

Lastly, the instrument itself can be better refined. The transfer lines can move solid particles, but an improvement is needed to prevent the lines from being partially or fully blocked by solid particles during the test. Better pumps for the transfer lines could be evaluated for more accurate flow rate calibrations. The current pumps are calibrated prior to each experiment to

determine the transfer rates, but they do not exactly meet the target transfer rates. A better engineered mechanism for controlling the travel distance of each cylinder would also help in making the instrument more robust from experiment to experiment. This could be accomplished using by fabricating a straight arm metal pole inside a secondary metal tube with a stop arm to allow the accurate travel distance of the sample cylinder itself.

Chapter 5. References

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