Monitoring Dissolution of Nonsteroidal Anti-Inflammatory Drugs Using Infrared Spectroscopy

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Monitoring Dissolution of Nonsteroidal Anti-Inflammatory Drugs Using Infrared Spectroscopy

by

Julianne Berger

THESIS

Submitted to the Department of Chemistry and Biochemistry at Seton Hall University in partial fulfillment of the requirements for the degree of Master of Science.

May, 2015
We certify that we have read this thesis and that in our opinion it is adequate in scientific scope and quality as a thesis for the degree of Master of Science.

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Abstract

The main purpose of this work was to demonstrate the use of infrared spectroscopy to follow the dissolution process of naproxen sodium. Tablets from three different vendors containing naproxen sodium were analyzed to address a common question, is there is a difference between a branded and generic product? Several aspects of that question are discussed in this work. These include comparing the appearance of each product, the rates of dissolution under conditions similar to different parts of the body and the dissolution kinetics.

A comparison study was conducted to examine the formulation of three brands of naproxen sodium. These brands were Bayer Aleve®, CVS All Day Pain Relief and Walgreens All Day Pain Relief. Bayer Aleve® is the brand name product whereas CVS All Day Pain Relief and Walgreens All Day Pain Relief are generic brands. The active and inactive ingredients were the same because generally the generic brand formulations mimic the branded product. The size and shape of the tablets differ among the three brands. Bayer Aleve® and CVS All Day Pain Relief are round in shape whereas Walgreens All Day Pain Relief is oval. Besides the shape, there were other components during the dissolution process that were noticeably different about the Walgreens All Day Pain Relief tablets. Lastly, S-naproxen was extracted from the tablets of each brand and evaluated by GC-MS to determine the purity of naproxen in the tablets. It was determined that the purity ranged between 97-98% which was comparable to the purity of the standard. The chromatograms identified two compounds, methyl naproxen and 4-methoxybiphenyl, in the tablet extracts of Bayer Aleve® and Walgreens All Day Pain Relief yet these compounds were not present in the CVS All Day Pain Relief extract.

Dissolution of naproxen was monitored by an IR probe under standard USP conditions. Each test lasted a total of eight hours using 0.1 M phosphate buffer pH 7.4, 0.05
M phosphate buffer pH 4.5 and simulated gastric fluid pH 1.2 as the medium. The percent dissolved was calculated for each brand. Times varied depending on the pH yet it was determined that the brands dissolved one hundred percent in an eight hour time span. While using 0.05 M phosphate buffer pH 4.5, there was a drop in baseline for Bayer Aleve ® and CVS All Day Pain Relief experiments which was caused by the fluctuation of temperature. Also, there was a shift in wavenumber of the peaks monitored as the medium became more acidic. This may be caused by protonation of the molecule as the medium became more acidic or the salt in the tablet was affected by the environment in which it was monitored. The peak energy may be dependent upon the composition of the tablet within solution.

Finally a kinetic study was completed for each brand at all three pH levels. It could not be determined if a first order process took place at the beginning of the dissolution process. This may be attributed to an intermediate step, an absorbing excipient present in the formulation or due to multiple processes that are occurring. However as time evolved the dissolution profile resembled a zero-order process.
1. Introduction

1.1 NSAIDs and Naproxen

Nonsteroidal anti-inflammatory drugs (NSAID) relieve pain and reduce fevers. These drugs have anti-inflammatory effects but are non-narcotic, unlike steroids. Three examples are naproxen, aspirin and ibuprofen. A NSAID has various medical uses and may be taken to relieve a headache, mild-to-moderate pain, muscle stiffness and rheumatoid and osteoarthritis. There are also many reasons to avoid taking NSAIDs which include but are not limited to people who have gastrointestinal disorder problems, irritable bowel syndrome, inflammatory bowel disease, cardiac disease, or are in the third trimester of pregnancy. NSAIDs can either be over-the-counter and/or prescription which are broken down into generic and name brand products. Some common brands include Celebrex, Motrin and Aleve.¹

Most nonsteroidal anti-inflammatory drugs are chiral molecules and weak acids. These molecules have a pKa between three and five and absorb nicely in the stomach and intestines because the pH of the stomach is very low. Each NSAID can be classified into a specific group by its chemical structure and/or its mechanism of action. These include salicylates, propionic acid derivatives, anthranilic acid derivatives, selective COX-2 inhibitors, acetic acid derivatives, sulphonanilides, enolic acid derivatives, natural and others. Within a group, the NSAIDs have similar characteristics such as structure yet will have differing dosages and administration routes.²

Aspirin is an example of a salicylate, whereas naproxen and ibuprofen are examples of propionic acid derivatives. Propionic acid (C₃H₆O₂) is a naturally occurring carboxylic acid whose sodium salt is a preservative. Naproxen falls in the propionic acid derivatives category because the structure of naproxen has the propionic acid chain and is distributed as
a sodium salt. By comparing Figure 1 with Figure 2, it can be confirmed why naproxen is a
NSAID within the propionic acid derivatives group.³

Naproxen is a chiral molecule with a stereogenic center; therefore it has R and S
enantiomers. The (S)-enantiomer is the biologically more active form since it is 28 times
more active than the (R)-enantiomer. As a result, naproxen was the first NSAID to be
marketed in its enantiomerically pure form. Naproxen is also known as (+)-(S)-2-(6-
methoxynaphthalen-2-yl) propionic acid and was first synthesized by Syntex in 1967
followed by clinical approval in 1972.⁴⁵

Naproxen and naproxen sodium are the main ingredients in some branded products,
such as Aleve, Naprelan, Flanax, Anaprox and Midol Extended Relief, to name a few.⁷
These drugs reduce pain, fever, inflammation and stiffness. Originally, naproxen was
marketed by Syntex in 1976 as a prescription drug called Naprosyn followed by the sodium
salt marketed as Anaprox in 1980. In 1994 the Food and Drug Administration approved
naproxen containing drugs to be purchased over-the-counter however, in other parts of the
world these drugs must be bought with a prescription. Once FDA approved, Bayer Health
Care took over most of the over-the-counter marketing and established their product, Aleve
and other generic store brand formulations. Most formulas containing naproxen include its
sodium salt.⁸

The purpose of taking Aleve is to temporarily prevent the body’s production of
prostaglandins which cause pain. That is why Aleve is an NSAID because it relieves pain
and reduces fevers. The active ingredient in Aleve is naproxen sodium (220 mg) which is
broken down into 200 mg of naproxen and 20 mg of its sodium salt. Inactive ingredients
include FD & C blue #2, hypromellose, magnesium stearate, microcrystalline cellulose,
Figure 1 – Propionic Acid and Sodium Propionate, respectively
Figure 2 – Naproxen and Naproxen Sodium, respectively
polyethylene glycol, povidone, talc and titanium dioxide. When taking Aleve, one tablet or capsule should be taken every eight to twelve hours with the possibility of taking two tablets or capsules within the first hour.\textsuperscript{9,10} However, three tablets or capsules in a twenty-four hour period should not be taken. Besides the warning just mentioned, there is plenty of drug information found on the products label.\textsuperscript{10}

The sodium salt component of naproxen was developed as a quicker absorbing formulation in order to relieve pain as fast as possible. Naproxen undergoes complete absorption in the gastrointestinal tract and has an \textit{in vivo} bioavailability of 95\%.\textsuperscript{12} The sodium salt of naproxen is the better absorbing of the two components because even a few milligrams of sodium aids in the body’s absorption of the drug. Absorption is very important when developing a drug because the drug must absorb before any of its affects can be seen. As a result, the pharmacokinetics of a drug can simply be changed by altering the absorption. Pharmacokinetics describes how the drug will affect a system once administered. Absorption and distribution are two aspects that pharmacokinetics monitors once a drug enters the body. Absorption relies on the type of release the drug undergoes while distribution depends on the amount of drug in the formula.

The effect that absorption and distribution of a drug has on the body is determined by a set of clinical trials before the drug is marketed to the public. These clinical trials provided information on the affects that naproxen had on patients with rheumatoid arthritis, osteoarthritis, tendinitis and acute gout. Signs of reduced joint pain as well as increased motion in knee joints were found with patients who have osteoarthritis. Here the patients who experience rheumatoid arthritis and osteoarthritis compared the affects of naproxen to aspirin.\textsuperscript{13}
1.2 Dissolution Testing

In the pharmaceutical industry, dissolution testing is widely used to provide *in vitro* drug release information. Dissolution testing monitors the absorption of a drug within a system and measures the rate of drug release in its dosage form. In other words, it is the breakdown of a solid into its components. The amount of solid dissolved depends on the thermodynamic energies involved in the system. The rate of dissolution is affected by solvent, solute, temperature, mixing of the system and surface area. These components help control the duration of the drug within the system. As a result, rates of the same active ingredient may not be comparable to each other if a component mentioned above is altered.

Drugs are formulated into tablets and capsules which usually contain other inactive ingredients called excipients. Some excipients are microcrystalline cellulose, magnesium stearate, and carboxymethylcellulose. The active ingredient is formulated with excipients to help bind a tablet together as well as a way to manage the taste of the product. Formulations can also help control the rate in which the drug is released. Therefore, dissolution testing is a tool that will help monitor the rate at which a drug is released from its binding. Dissolution of a tablet or capsule as explained above, involves the breakdown of a drug into smaller particles. Dissolution occurs with the aid of a phosphate buffer (the medium) that simulates a bodily fluid at a certain pH level with constant mixing and constant temperature. Therefore, the rate of dissolution will depend on three factors: granule size of the drug, structure and formulation of the tablets or capsules and pH of the medium.\textsuperscript{14}

A tablet or capsule must be in solution for a drug to be absorbed within the body. The rate at which the drug dissociates is crucial for pharmacokinetic and pharmacodynamic properties. An example of this is solubility, which can be broken down into two categories,
equilibrium and kinetic. Solubility can help determine the absorption of a drug. It depends on the chemical and physical properties of the substance as well as the temperature and pH of the solution. Equilibrium solubility takes place when a compound in a saturated solution comes in contact with an excess of undissolved solid yet kinetic solubility occurs when a precipitate appears due to a change in concentration or a change in polarity of the solvent.\textsuperscript{15}

1.3 Thermodynamics and Kinetics of Dissolution

Dissolution and solubility have different meanings like kinetics and thermodynamics. Kinetics relates to dissolution while thermodynamics relates to solubility. Thermodynamics is the relationship between heat and energy which can be converted into work. Heat is the energy transfer from one system to another from high to low temperature. Work is the energy interaction between a system and its surroundings. There are three laws of thermodynamics which are defined by temperature, energy and entropy of a system. A system is a distinct region that is separate from the surroundings. The surroundings can do work on the system or the system can do work on its surroundings. This is demonstrated in Equations 1 and 2.

\[
\Delta E = q + w \quad \text{Equation (1)}
\]

\[
\Delta S_{\text{universe}} > 0 \quad \text{Equation (2)}
\]

The second law of thermodynamics involves a system heat transfer. Basically heat cannot move from cold to hot. This law also explains that the entropy of the universe never decreases for an irreversible process if the system is isolated, as seen in Equations 3-5. Entropy (S) is a measure of disorder within a thermodynamic system. When related to dissolving a substance, the change in entropy can either be endothermic or exothermic. A
process will be endothermic if the energy to break the bonds between cations and anions is larger than the energy it takes to form bonds between the solute and solvent. Here the change in entropy is negative. For a process to be exothermic, the opposite occurs. The amount of energy needed to break cation and anion bonds is smaller than the energy it takes to form new bonds between the solute and solvent. In this case, the change in entropy is positive.\textsuperscript{16, 17, 18}

\[
\Delta S_{\text{univ}} = \Delta S_{\text{sys}} + \Delta S_{\text{surr}} \quad \text{Equation (3)}
\]
\[
\Delta S = q/T \quad \text{Equation (4)}
\]
\[
\Delta S_{\text{univ}} = (q_{\text{sys}}/T) + (q_{\text{surr}}/T) \quad \text{Equation (5)}
\]

Thermodynamics and free energy is the amount of work that a system can perform at a given time. Free energy is the energy of a system that is converted into work. There are two types of free energy, Helmholtz free energy and Gibbs free energy. Helmholtz free energy is the energy that can be converted into work at a constant temperature and volume. Gibbs free energy is the energy that can be converted into work at a constant temperature and pressure throughout an entire system. Gibbs free energy is the change in enthalpy of the system minus the product of the temperature and the change in entropy, shown in Equation 6. Entropy and enthalpy are what drive the reaction. A reaction will be thermodynamically favorable if the change in Gibbs free energy (\(\Delta G\)) is negative. As a result, the entropy of the system will increase causing the change in entropy (\(\Delta S\)) to be positive so the change in enthalpy (\(\Delta H\)) will be negative. This is an example of a product-favored reaction. If the opposite occurs, change in Gibbs free energy (\(\Delta G\)) will be positive, change in enthalpy (\(\Delta H\)) will also be positive and change in entropy (\(\Delta S\)) will be negative. Here the reaction does not take place on its own therefore it is a non-spontaneous reaction.\textsuperscript{16, 17, 18}
\[ \Delta G = \Delta H - T\Delta S \]  

Equation (6)

Kinetics is the study of the rate of a chemical reaction. Chemical kinetics of a reaction is affected by experimental conditions that influence the speed of a reaction. Some factors that affect the reaction rate include the nature of the reactant(s), the physical state of the reactant(s), the concentration, the temperature, the pressure and catalysts. A kinetic profile will determine if the reaction is thermodynamically and kinetically favorable. These two components determine if the reaction will occur. Mathematical models help illustrate these characteristics and help determine the reaction rate for a reaction using rate laws and rate constants. A rate law is an Equation that combines the reaction rate with concentration or pressures of the reactant(s). There are three common rate laws: zero-order, first order and second order. A zero-order reaction is independent of the concentration of the reactant(s). A first order reaction depends on the concentration of one reactant whereas a second order reaction depends on the concentration of one second order reactant or two first order reactants. The integrated rate laws for all three orders are listed in Equations 7-9, respectively.17, 18, 19

\[
[A]_t = -kt + [A]_0 \quad \text{Equation (7)}
\]
\[
\ln [A] = -kt + \ln [A]_0 \quad \text{Equation (8)}
\]
\[
1/[A] = 1/[A]_0 + kt \quad \text{Equation (9)}
\]

Dissolution is a kinetic process because as time evolves concentration is either increasing or decreasing with the rate of the reaction over a period of time. These rate laws can verify the kinetic order of a reaction by using the concentration and the reaction rate of each system. However depending on the reactant, the reaction rate will vary. A reaction will tend to be slower if strong intermolecular interactions form between the solute and the
solvent. The rate of a reaction will also be affected by the strength of the bonds of the reactant; the greater the bond strength, the slower the reaction. Although, concentration and temperature would typically affect the reaction rate the most. For dissolution testing, concentration is dependent on the drug itself and how it reacts with the solvent. As the tablet begins to dissolve, molecules collide with one another. More collisions would occur if the concentration was increased. Temperature on the other hand remains constant throughout dissolution testing. Molecules will collide faster at higher temperatures as well as if the temperature was increased. Besides concentration and temperature, there are other factors that affect the rate of a reaction during dissolution.\textsuperscript{17, 18, 19}

Naproxen has two components, immediate release and sustained release.\textsuperscript{20} Immediate release is the instant release of the drug after administered to the body. Many drugs are designed to release immediately, such as pain killers. These drugs start working within minutes to hours as the tablet disintegrates. This is an example of a first order reaction because the drug is released in a single action and the highest plasma level or concentration maximum is reached in a short period of time.\textsuperscript{21} Thirty percent of naproxen sodium is an immediate release where plasma levels can be detected after thirty minutes of taking the drug.\textsuperscript{20} The other component of naproxen is a sustained release dosage which takes place after a period of time. This type of release occurs throughout the entire GI tract and would ultimately reduce dosage amounts. Sustained release tablets typically have a polymer coating which is known as a reservoir system and follows zero-order kinetics.\textsuperscript{21} These two release systems cannot occur at the same time because one component is an instant release of the drug while the other component will release the drug after a prolonged period of time.
1.4 Overview

The main purpose of this study was to monitor by Fourier Transform Infrared Spectroscopy (FTIR) the rate at which naproxen dissolved by dissolution testing. This was performed using a new instrument: ReactIR. A silver halide probe was inserted into the dissolution vessel where it took IR readings every two minutes. These absorbances were located in the fingerprint region (1400 cm$^{-1}$ to 600 cm$^{-1}$) of the IR spectrum. Here bonds are excited to higher energy states and are typically caused by stretching and bending motions of diatomic units. These include carbon-carbon bonds (a ring or straight carbon chain), carbon-oxygen bonds (alcohol, carboxylic acid, or ether), and carbon-nitrogen bonds (amine and amides). For example, the carbon-oxygen stretch for an ether absorbs between 1000-1300 cm$^{-1}$. When choosing a wavenumber to monitor it should correlate to functional groups that are specific for naproxen. In this study, the carbon-oxygen bond was chosen as the functional group of interest and monitored by ReactIR. Using the absorbances taken by the probe and ReactIR, the percent dissolved was calculated for each tablet within an eight hour period. Next the dissolution profiles were examined further by conducting a kinetic study. First order and zero-order kinetic plots were evaluated since naproxen sodium tablets are immediate release and sustained release systems.

The remaining chapters explain in further detail the main points which were mentioned above. The first part of this work compared the packaging and formulation of three brands of naproxen. The content uniformity was evaluated to determine the purity of naproxen for each brand by GC-MS. The second part of this work was a dissolution study that examined three different brands of naproxen sodium at three different pH levels. During dissolution, a wavenumber was monitored that was specific to the naproxen molecule. Using the absorbances collected by the ReactIR, the percent dissolved was calculated for each
brand. Here one tablet was dropped and monitored by an IR probe over the course of eight hours. As the pH became acidic, the baseline began to drop and the wavenumber shifted. Lastly, a kinetics study was conducted for a first order and zero-order reaction. A first order reaction could not be distinguished during dissolution testing because multiple processes are occurring as the tablet dissolved.
2. Experimental Section

2.1 Materials

Reagents (monobasic sodium phosphate, anhydrous dibasic sodium phosphate, potassium phosphate monobasic, sodium chloride and magnesium sulfate) and solvents (hydrochloric acid, acetone and ethyl acetate) were purchased from Sigma-Aldrich, Fisher Scientific, Sigma Chemical, Mallinckrodt Chemicals, and Macron Chemicals, respectively. S-naproxen was bought from Fluka (lot 1197390). All three naproxen brands were bought at a local drug store, Aleve ® (lot NAAJTEP), CVS All Day Pain Relief (lot 3CE1694A) and Walgreens All Day Pain Relief (lot 3JE1957B). During the extraction of S-naproxen from tablets, filtering was achieved using a Buchner Funnel and Whatman #2 filter paper (90 mm). Ethyl acetate used during extraction was evaporated using a Buchi Heating Bath B-490 and a Buchi rotavapor R-200. Dry ice, used in the Buchi rotavapor, was supplied by Seton Hall University.

2.2 Procedure for Extracting S-Naproxen from Tablets

Ten tablets were dissolved in 100 mL of distilled water. These tablets were soluble in water and dissolved after a few minutes forming a blue solution. Once dissolved, the blue solution was filtered using a Buchner funnel and Whatman #2 filter paper to remove any undissolved salts. At this point, the blue naproxen solution was in its sodium salt form. The pH of the solution was adjusted by adding dilute HCl until the blue solution became cloudy. The dilute HCl transformed the sodium salt into a free base (cloudy solution). This was followed by extracting the cloudy solution with 3x100 mL of ethyl acetate. At the end of the three extractions, a white crystal-like substance formed in the collected layer. The solution
was passed through a vacuum filter and mixed with anhydrous MgSO₄ to remove any water that was present.

This solution was then transferred to a 500 mL round bottom flask and attached to a rotary evaporator. Dry ice and a little heat aided in the evaporation of the ethyl acetate. Once the solution was evaporated, a white powdery solid appeared on the round bottom flask. The white solid was then scrapped out of the round bottom flask and weighed. The label claim for naproxen sodium per tablet was 220 mg therefore; the theoretical yield for ten tablets was 2.2 g of S-naproxen.

2.3 Procedure for 0.1 M Phosphate Buffer pH 7.4
0.1 M phosphate buffer was prepared with monobasic sodium phosphate and dibasic sodium phosphate as per USP procedures.²⁴ 2.6 g of monobasic sodium phosphate and 11.5 g of dibasic sodium phosphate were dissolved in 1000 mL of distilled water. The pH of the solution was checked and adjusted if needed.

2.4 Procedure for 0.05 M Phosphate Buffer pH 4.5
0.05 M phosphate buffer was prepared with potassium phosphate monobasic as per USP procedures.²⁵ 6.8 g of potassium phosphate monobasic was dissolved in 1000 mL of distilled water. The pH of the solution was checked and adjusted if needed.

2.5 Procedure for Simulated Gastric Fluid USP pH 1.2
Simulated gastric fluid was prepared with sodium chloride and hydrochloric acid as per USP procedures.²⁶ 2.0 g of sodium chloride was dissolved in 300 mL of distilled water. Once
dissolved, 7.0 mL of hydrochloric acid was added to the solution. After stirring, 700 mL of distilled water was added to the solution to make a 1000 mL solution. The pH of the solution was checked and adjusted if needed.

2.6 Procedure for Naproxen Standard

220 mg of S-naproxen were dissolved in 100 mL of 0.1 M phosphate buffer, 0.05 M phosphate buffer or simulated gastric fluid.

2.7 Instrumentation

Dissolution testing was completed using a combination of two instruments, ReactIR iC10 and EasyMax 102 Synthesis Workstation. Its main function was to monitor how a reaction evolved over a period of time using FTIR. The FTIR analysis was taken by a silver halide (AgX) fiber optic probe that was attached to the ReactIR, shown in Figures 3 and 4. The probe was inserted into the dissolution vessel where it scanned the solution every two minutes. These scans correspond to absorbances found in the fingerprint region of the infrared spectrum. The ReactIR is equipped with a MCT detector and a Fiber Conduit. The MCT detector is cooled by liquid nitrogen and the Fiber Conduit is IR transparent with silver chloride/silver bromide optical fibers. Also, the fiber optic probe is a 1mm diamond coated ATR probe with an 8 wavenumber resolution.22, 27

2.8 Procedure for Operating the EasyMax and ReactIR

Using the EasyMax control pad, the instrument was powered up and a 100 mL dissolution vessel was placed in reactor 2 with the top cover locked in place. The
The ReactIR is designed to occupy small spaces either in a fume hood or on a bench top. It can be used in any laboratory to provide a variety of information for a reaction. It is connected to a silver halide fiber optic probe which monitors absorbance’s in the fingerprint region of the infrared spectrum.


There are four types of IR probes that can be connected with the ReactIR iC10. For these experiments, a silver halide fiber optic probe was chosen with a 9.5 mm diameter that was attached to a fiber conduit. The fiber optic probe has a pressure limit of 69 bar and can be used under temperature conditions ranging up to 180°C.

http://at.mt.com/at/de/home/supportive_content/product_documentation/datasheets/ReactIR_iC10_DS_090309.pdf

thermometer and the electronic stirrer were located on the top cover. Once the apparatus was set up, 100 mL of phosphate buffer or simulated gastric fluid was added to the vessel via an opening on the cover. The stirrer speed and temperature of the vessel were activated by using the control pad at 50 rpm and 37°C, respectively. Figure 5 displays this setup. Next, a new experiment was programmed using the iC IR software on the computer. The duration of each experiment was set to eight hours and the scans taken by the IR probe was chosen to occur every two minutes. Liquid nitrogen was added to the top of the ReactIR until it began to overflow. The liquid nitrogen acted as a cooling agent for the MCD detector and helped align the instrument. Alignment was one hundred percent if one or both bars on the screen turned green (Appendix 1). The changing of any mirrors found in the instrument would affect the alignment of the probe and change the flow of the instrument. Background noise may also affect the flow of the instrument.

The fiber optic probe was then cleaned using a kimwipe along with a little acetone and distilled water. Before the experiment began, a reference background was collected as well as scans of any reference samples. These references included the phosphate buffer or simulated gastric fluid (solvent) and the standard (reactant). Lastly, the fiber optic probe was cleaned and inserted into the top cover of the dissolution vessel where it was clamped at the perfect height inside the vessel. Again, Figure 5 demonstrates this setup and step by step instructions can be found in Appendix 1.

Each experiment was started by clicking the play button on the main screen of the software. After twenty minutes, one tablet was dropped into the vessel by way of the top cover. As the experiment evolved, all of the scans were collected and saved to the software. These results were monitored as the dissolution testing took place and after completion of the
Figure 5 – Setup of Mettler Toledo EasyMax 102 Synthesis Workstation
experiment. The probe was loosened and placed outside of the EasyMax workstation at the end of the eight hours. Lastly, the vessel was cleaned and the temperature of the reactor was adjusted to 20°C using the control pad.

2.9 Procedure for Extracting Data from iC IR Software

The main screen of the software was broken down into four sections. For data analysis purposes, the event tab, spectra tab and trend tab were utilized the most. All scans taken over the eight hour period could be found in the event tab. Each scan was individually pinned by double clicking the pin icon on the left of the screen. Once pinned, these readings appeared in the spectra tab. Within the spectra tab, a peak was isolated among all of the spectra by choosing the peak tab on the far left. This specific peak was 1217 cm⁻¹ and a two-point baseline was used to calculate the area of the peak. The peak range was 1228-1205 cm⁻¹ with a baseline of 1235-1200 cm⁻¹. Next, the calculated peak was subjected to mathematical smoothing by choosing user-defined trends within the trend tab on the right of the screen. Finally, the smoothing data was copied to Excel for further analysis.

2.10 Dissolution: Traditional versus ReactIR and EasyMax Workstation Combination

The combination of ReactIR and EasyMax Synthesis Workstation was much different than traditional dissolution testing apparatuses; however some components were the same. Traditional dissolution testing provides in vitro information involving 900 mL of a medium at a temperature of 37°C and constant stirring at 50 rpm. The medium is stirred either by a basket (Apparatus 1) or a paddle (Apparatus 2). For Apparatus 1, the tablet is placed inside the basket for the duration of the experiment however the tablet is dropped into solution
when using Apparatus 2, as seen in Figure 6. When using the combination of ReactIR and EasyMax Workstation, a fiber optic probe was inserted into a 100 mL vessel and was heated to 37°C with constant stirring, set to 50 rpm. Each experiment lasted for eight hours which was programmed on the iC IR software. An eight hour period was chosen as the length of time for each experiment because the directions found on the label state to take one tablet every eight hours. Once the experiment started, one tablet was dropped into solution after twenty minutes of equilibration. All experiments were executed the same way under these conditions which can be found in Table 1.

2.11 pH Levels

Bayer Aleve ®, CVS All Day Pain Relief and Walgreens All Day Pain Relief were exposed to three different pH levels which mimic different parts of the body. The 0.1 M phosphate buffer (pH 7.4) mimics saliva, 0.05 M phosphate buffer (pH 4.5) replicates the duodenum which is the upper part of the small intestine and lastly, simulated gastric fluid (pH 1.2) mimics the lower stomach. The percent dissolved was calculated for each brand at all three pH levels using fifteen different time points over an eight hour period. It was observed that the percent dissolved for each brand at 1217 cm⁻¹ exemplified a dissolution profile where one hundred percent release of the drug from the tablet occurred. As the tablet was dropped into solution, the drug began to release at a rapid pace before it leveled off. It was concluded that between the three different pH levels, one hundred percent of the drug was dissolved for each brand. As the pH became more acidic, the appearance of the dissolution profile began to change. Also, the amount of time for the drug to dissolve one hundred percent varied between brands and pH levels.
## Dissolution Conditions

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medium</strong></td>
<td>100 mL buffer</td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td>8 hours</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>37°C</td>
</tr>
<tr>
<td><strong>Stirrer Speed</strong></td>
<td>50 rpm</td>
</tr>
<tr>
<td><strong>Equilibration Time</strong></td>
<td>20 minutes</td>
</tr>
</tbody>
</table>

Table 1 – Dissolution Conditions for Naproxen Experiments
Figure 6 – Traditional Dissolution: Apparatus 1 and Apparatus 2, respectively
2.12 Kinetic Study

A kinetic study for all three brands at each pH level was evaluated. Using the absorbances taken by the IR probe, the concentration was calculated using Beer-Lambert law. This states that the relationship between absorbance and concentration of an analyte is linear. The Equation listed below explains this relationship. Absorbance is equal to the product of the molar absorptivity, the cell length and the concentration of the analyte. For purposes here, the cell length was equal to 0.95 cm (diameter of the IR probe) and the molar absorptivity was equal to the slope of the line from the appropriate calibration curve. Using those values just mentioned and the absorbances acquired during each experiment, the concentration was calculated by dividing the absorbances by the slope and the cell length. This concentration was used to determine the kinetic order for each brand.

\[ A = \varepsilon bc \quad \text{Equation (8)} \]

For a kinetic plot, the rate constant, k (s\(^{-1}\)), is the decrease in concentration of the reactant per time. It also determines the speed of the reaction, how fast or how slow the drug will be consumed in the medium. As a result, the slope (k) of a first order kinetics plot is negative. A zero-order kinetics plot (concentration versus time) should also produce a negative slope and was generated for all three brands at all three pH levels. As time evolved, the amount of concentration extracted from the drug increased and leveled off when an equal amount of the drug was released. This typically occurred at the end of each experiment where the reaction was independent of concentration and produced a straight line. First order and zero-order kinetics was evaluated because naproxen sodium is comprised of two components, immediate release and sustained release.
Results and Discussion

3.1 Physical Properties/Comparison

The label and appearance of Bayer Aleve®, CVS All Day Pain Relief and Walgreens All Day Pain Relief were compared. It was noted that the three brands have many similarities, however there are some differences. Table 2 compares and contrasts the branded product and the two generic brands. Besides the active ingredient, the three products also have the same inactive ingredients, label claim and dosage to name a few. The highlighted boxes denote the differences between the three brands which include the shape and size of the tablet as well as the amount of sodium present in the tablet. The label claim for all three brands is 220 mg of naproxen sodium, which is comprised of 200 mg of naproxen and 20 mg of sodium (salt). The salt component is considered low sodium by FDA guidelines. When the components were broken down on the label, only Bayer Aleve® listed sodium as 20 mg. The labels for CVS All Day Pain Relief and Walgreens All Day Pain Relief list sodium as 21 mg which would make the label claim 221 mg instead of 220 mg. The different shape and size among the three products was expected because it acts as an identity for the product.

The packaging was similar for the CVS All Day Pain Relief and Walgreens All Day Pain Relief brands, however, the packaging for Bayer Aleve® was more consumer oriented. This proved that CVS All Day Pain Relief and Walgreens All Day Pain Relief were generic brands of Bayer Aleve®. Besides the packaging, generic brands should be relatively consistent with the branded product. In conclusion, the only difference between a branded product and its generics should be the shape and size of the tablet as well as the packaging. This was relevant when comparing Bayer Aleve® to CVS All Day Pain Relief and Walgreens All Day Pain Relief.
<table>
<thead>
<tr>
<th>Brand</th>
<th>Aleve ®</th>
<th>CVS All Day Pain Relief</th>
<th>Walgreens All Day Pain Relief</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active Ingredient</strong></td>
<td>Naproxen Sodium</td>
<td>Naproxen Sodium</td>
<td>Naproxen Sodium</td>
</tr>
<tr>
<td><strong>Label Claim</strong></td>
<td>220 mg</td>
<td>220 mg</td>
<td>220 mg</td>
</tr>
<tr>
<td><strong>Active Ingredients Breakdown</strong></td>
<td>200 mg of Naproxen and 20 mg of Sodium</td>
<td>200 mg of Naproxen and 21 mg of Sodium</td>
<td>200 mg of Naproxen and 21 mg of Sodium</td>
</tr>
<tr>
<td><strong>Inactive Ingredients</strong></td>
<td>FD&amp;C blue #2 lake, Hypromellose, Magnesium Stearate, Microcrystalline Cellulose, polyethylene glycol, providone, talc and Titanium Dioxide</td>
<td>FD&amp;C blue #2 aluminum lake, Hypromellose, Magnesium Stearate, Microcrystalline Cellulose, polyethylene glycol, providone, talc and Titanium Dioxide</td>
<td>FD&amp;C blue #2 aluminum lake, Hypromellose, Magnesium Stearate, Microcrystalline Cellulose, polyethylene glycol, providone, talc and Titanium Dioxide</td>
</tr>
<tr>
<td><strong>Shape</strong></td>
<td>Round</td>
<td>Round</td>
<td>Oval</td>
</tr>
<tr>
<td><strong>Size</strong></td>
<td>14 mm</td>
<td>10 mm</td>
<td>12 mm</td>
</tr>
<tr>
<td><strong>Product Type</strong></td>
<td>OTC</td>
<td>OTC</td>
<td>OTC</td>
</tr>
<tr>
<td><strong>Route of Administration</strong></td>
<td>Oral</td>
<td>Oral</td>
<td>Oral</td>
</tr>
<tr>
<td><strong>Purpose</strong></td>
<td>Pain reliever/fever reducer</td>
<td>Pain reliever/fever reducer</td>
<td>Pain reliever/fever reducer</td>
</tr>
<tr>
<td><strong>NSAID</strong></td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td><strong>Dosage</strong></td>
<td>1 tablet every 8-12 hours. The first dose you may take 2 tablets.</td>
<td>1 tablet every 8-12 hours. The first dose you may take 2 tablets.</td>
<td>1 tablet every 8-12 hours. The first dose you may take 2 tablets.</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>20-25°C</td>
<td>20-25°C</td>
<td>20-25°C</td>
</tr>
<tr>
<td><strong>Distributor</strong></td>
<td>Bayer HealthCare</td>
<td>CVS</td>
<td>Walgreens</td>
</tr>
</tbody>
</table>

Table 2 – Label Comparison between Bayer Aleve ®, CVS All Day Pain Relief and Walgreens All Day Pain Relief
3.2 IR and GC-MS Results from Standards

3.2.1 Content Uniformity

Content uniformity testing was performed to ensure there was no inconsistency between tablets within a batch. This inconsistency usually includes the active ingredient. Therefore this potential of variability between tablets was evaluated by isolating the active ingredient, S-naproxen, from the tablets. In order to extract S-naproxen from the tablets, a procedure was followed from Erin Sharp’s honors thesis. This procedure can be found in the experimental section. By extracting the active ingredient, the purity of each brand could be estimated by GC-MS.

Ten tablets were dissolved in water and as the tablets lost its coating the solution turned blue. Dilute hydrochloric acid was added to this blue solution which turned the solution cloudy. This was a result of the sodium salt becoming a free base. With the aid of dry ice, acetone and heat (40°C), the S-naproxen was extracted by ethyl acetate and isolated by a rotary evaporator. When all of the ethyl acetate evaporated, a crystal-like powder appeared at the bottom of the round bottom flask. This crystal-like substance was collected and analyzed by an outside laboratory (Advanced Biotech) using GC-MS. The amount of S-naproxen extracted from each brand can be found in Table 3. The label claim for ten tablets is 2.2 g of S-naproxen. The amount of S-naproxen recovered from the Bayer Aleve® tablets was the largest, 84.2% of the label claim where as for CVS All Day Pain Relief the smallest amount of S-naproxen was recovered, 35.5% of the label claim. CVS All Day Pain Relief tablets had the smallest percent extracted because the active ingredient was not fully extracted from the tablets. The percent extracted for Bayer Aleve® and Walgreens All Day Pain Relief are much higher and very consistent with each other.
<table>
<thead>
<tr>
<th>Product</th>
<th>Amount Extracted (g)</th>
<th>Percent Extracted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayer Aleve ®</td>
<td>1.68 g</td>
<td>84.2%</td>
</tr>
<tr>
<td>CVS All Day Pain Relief</td>
<td>0.71 g</td>
<td>35.5%</td>
</tr>
<tr>
<td>Walgreens All Day Pain Relief</td>
<td>1.63 g</td>
<td>81.4%</td>
</tr>
</tbody>
</table>

Table 3 – Percent Recovery of S-naproxen from Tablets
Using the amounts of S-naproxen from Table 3, the purity of naproxen for each brand was estimated by GC-MS. The GC-MS work presented in this section was completed by Tom DelMastro at Advanced Biotech. The chromatogram in Figure 7 is an overlay for the extracts of Bayer Aleve®, CVS All Day Pain Relief and Walgreens All Day Pain Relief. Appendix 2 lists the sum of corrected peak areas for each brand. Between the three products, a few peaks overlap each other. The first peak at a retention time of 4.1 minutes is ethyl acetate. The presence of ethyl acetate in all three brands indicated that some ethyl acetate did not evaporate during the rotary evaporator step of the extraction procedure. The amount of ethyl acetate was very small in Bayer Aleve® and Walgreens All Day Pain Relief. However there was a larger amount of ethyl acetate, 2.1%, present in the CVS All Day Pain Relief extract. This was most likely the result of a smaller amount of S-naproxen that was extracted from the tablets. Therefore, this was another indication that S-naproxen was not extracted fully from the CVS All Day Pain Relief tablets. The peak present at 20.8 minutes on the chromatogram is the S-naproxen. The purity of S-naproxen for all three brands is listed in Table 4 and ranges between 97% and 98%. These results were comparable to the purity of the naproxen standard (Fluka) which was 98%. The lower purity for the CVS All Day Pain Relief could be another suggestion that not all of the S-naproxen was initially extracted. Therefore if all of the S-naproxen was extracted, similar results to the Bayer Aleve® and Walgreens All Day Pain Relief samples would be expected.

The chromatogram shows a peak at a retention time of 19.8 minutes for Bayer Aleve® and Walgreens All Day Pain Relief. This retention time had a relative peak area that corresponded to the compound methyl naproxen where less than 0.5% was present in both extracts. Methyl naproxen has a very similar structure to naproxen however where the
<table>
<thead>
<tr>
<th>Product</th>
<th>Purity of S-naproxen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayer Aleve ®</td>
<td>98.4%</td>
</tr>
<tr>
<td>CVS All Day Pain Relief</td>
<td>97.9%</td>
</tr>
<tr>
<td>Walgreens All Day Pain Relief</td>
<td>98.6%</td>
</tr>
</tbody>
</table>

Table 4 – Purity of S-naproxen in Each Brand
Figure 7 - GC-MS Chromatogram of S-Naproxen Samples (Advanced Biotech)
hydroxyl group is present in the carboxylic acid chain, there is a methyl ester instead. There was another peak present for both Bayer Aleve ® and Walgreens All Day Pain Relief extracts. It was located at 16.2 minutes and was estimated to be the compound 4-methoxybiphenyl. There was less than 1.0% of this compound present in the Bayer Aleve ® and Walgreens All Day Pain Relief extracts. This compound, 4-methoxybiphenyl, is a white crystalline powder and is used as a flavoring agent. Both compounds just mentioned, shown in Figure 8, were not located in the CVS All Day Pain Relief extract. This could be another indication that the S-naproxen was not fully extracted or these two compounds were impurities that the ethyl acetate removed during extraction.

3.2.2 IR Spectral Analysis

Fourier Transform Infrared Spectroscopy is a method used to attain an infrared absorption spectrum of a solid, liquid or gas. This technique helps to determine how much light is being absorbed at a certain wavenumber. FTIR can help identify chemical compounds in a wide range of products and industries. An absorption spectrum helps identify compounds by detecting functional groups and bond information within the IR spectroscopy range, 4000-600 cm⁻¹. An IR spectrum of the naproxen standard was generated to learn a little bit more about the compound. The IR work presented in this section was completed by Tom DelMastro at Advanced Biotech. The IR spectrum in Figure 9 displays the percent transmittance of three distinct peaks to the left of the spectrum and a large number of peaks to the right of the spectrum. On the left there are three peaks: 3148.65 cm⁻¹ which corresponds to the O-H functional group part of a carboxylic acid, and 2976.41 cm⁻¹ and 2939.06 cm⁻¹ which are strong stretches from the C-H functional group. These
Figure 8 – GC-MS Chromatogram Containing Methyl Naproxen and 4-Methoxybiphenyl
Figure 9 – IR Spectrum of Naproxen Standard (Advanced Biotech)
absorbances were a result of the propionic acid chain found on the naproxen molecule. Other IR functional groups related to naproxen are listed in Table 5.\textsuperscript{31,32}

Looking at the IR spectrum, there are numerous peaks in the fingerprint region which are specific to naproxen. One band in particular is the C-O functional group which can either be related to a carboxylic acid or an ether. These absorptions are listed in Table 6.\textsuperscript{31,32} As a result, the fingerprint region became the area of interest especially because the ReactIR monitors absorbances in this region (1400 cm\(^{-1}\) to 600 cm\(^{-1}\)). It was probable that naproxen was a good molecule to observe using the ReactIR because of numerous peaks found in this region, which is shown in Figure 10.

When choosing a wavenumber to monitor, it is essential that it is specific to the molecule of interest. Interferences may include the components found in the medium or the excipients within the formulation of the tablet. In this study, phosphate buffer and simulated gastric fluid were used. These solutions will also absorb in the fingerprint region. For example, phosphate absorbs between 1100-1200 cm\(^{-1}\) and is a strong stretching vibration. Keeping that in mind, it was important to differentiate between peaks of the molecule and peaks from the medium or any other interference. If that wavenumber corresponded to a functional group specific for the active ingredient then this was a possible wavenumber to monitor.

3.3 \textit{pH 7.4 Dissolution Study}

\textbf{3.3.1 Calibration/LOD/LOQ}

A calibration curve was generated using 0.1 M phosphate buffer as the medium. A stock solution (5 mg/mL) and seven dilutions with different concentrations were monitored
Table 5 – IR Absorption Frequencies of Naproxen Functional Groups

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Functional Group</th>
<th>Absorptions (cm(^{-1}))</th>
<th>Intensity and Vibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxylic Acid</td>
<td>O-H</td>
<td>2500-3300</td>
<td>Strong Stretch</td>
</tr>
<tr>
<td>Alkane</td>
<td>C-H</td>
<td>2850-3000</td>
<td>Strong Stretch</td>
</tr>
<tr>
<td>Aromatic</td>
<td>C=C</td>
<td>1400-1600</td>
<td>Medium Stretch</td>
</tr>
<tr>
<td></td>
<td>C-H</td>
<td>3000-3100</td>
<td>Medium Stretch</td>
</tr>
<tr>
<td>Functional Group</td>
<td>Functional Group</td>
<td>Absorptions (cm$^{-1}$)</td>
<td>Intensity and Vibration</td>
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<tr>
<td>------------------</td>
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<td>-------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Carboxylic Acid</td>
<td>C-O</td>
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<td>Strong Stretch</td>
</tr>
<tr>
<td>Ether</td>
<td>C-O</td>
<td>1000-1300</td>
<td>Strong Stretch</td>
</tr>
</tbody>
</table>

Table 6 – Fingerprint Region IR Absorption Frequencies of Naproxen Functional Groups
Figure 10 – IR Spectrum of Fingerprint Region of Naproxen Standard (Advanced Biotech)
by the ReactIR. The area under the curve (AUC) was calculated for peaks present in naproxen solution yet absent in the medium. Peaks were present at a few wavenumbers; one in particular, 1217 cm\(^{-1}\). This peak is a stretching vibration which could be attributed to an alcohol, carboxylic acid or ether. The C-O functional group is a strong stretch typically found between 1320-1000 cm\(^{-1}\) on an IR spectrum. In the naproxen molecule, there is a carboxylic acid and an ether. The AUC for the peak present at 1217 cm\(^{-1}\) formed a straight line under 0.1 M phosphate buffer conditions which is shown in Figure 11. A blank displayed in Figure 12 helped determine if the chosen wavenumber was valid.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated to determine the smallest concentration of analyte that can be detected within a sample. Limit of detection is the minimal concentration that can be distinguished from the blank or the sample where as the limit of quantification is the lowest concentration that could be detected at a certain level of precision and accuracy. The limit of quantification is larger or equal to the limit of detection. The LOD and LOQ for the concentration of the analyte, which are listed in Table 7, can be calculated using Equations 9 and 10.\(^{35,36}\) The line fit plot for the calibration curves can be found on pages 84-85. It is important to remember that the analyte was present in every sample even if its concentration is lower than the LOD. This means that the analyte in that sample cannot be detected under these conditions because some sort of interference was occurring. For pH 7.4, the concentrations of all three brands are larger than the LOD.

\[
\text{LOD}_c = \frac{\text{Instrument LOD} - \text{Intercept}}{\text{Slope}} \quad \text{Equation (9)}
\]
\[
\text{LOQ}_c = \frac{\text{Instrument LOQ} - \text{Intercept}}{\text{Slope}} \quad \text{Equation (10)}
\]
Figure 11 – Naproxen Calibration Curve at pH 7.4

The graph shows a linear relationship between AUC and concentration, with the equation:

\[ y = 0.0293x + 0.1964 \]

and a goodness of fit of

\[ R^2 = 0.9928 \]
Figure 12 – 0.1 M Phosphate Buffer pH 7.4
<table>
<thead>
<tr>
<th>Medium</th>
<th>Limit of Detection (LOD) Concentration (mg/mL)</th>
<th>Limit of Quantification (LOQ) Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M Phosphate Buffer</td>
<td>0.009</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Table 7 – Concentration LODs and LOQs at pH 7.4
3.3.2 Percent Dissolved

Three experiments were generated for each brand using 0.1 M phosphate buffer. At a pH of 7.4, each brand dissolved one hundred percent within eight hours. However, the average rate of dissolution was different for each brand as seen in Table 8. Bayer Aleve ® dissolved one hundred percent just before eight hours whereas CVS All Day Pain Relief and Walgreens All Day Pain Relief dissolved one hundred percent a few hours earlier. The dissolution profiles for each brand can be found in Figure 13. There was slight variation between experiments for each brand over the eight hour period. This error was attributed to the instrument equilibration time or background noise. The variations shown in Figure 13 are displayed by error bars which become smaller and disappear as the experiments continued. The Bayer Aleve ® experiments have the most variation among the three brands.

3.3.3 Kinetic Study

Using the absorbance extracted from the iC IR software and the slope of the line produced from the calibration curve, the concentration can be calculated using Beer Lambert law. Using the calculated concentration, it was plotted versus time in seconds. The plot of absorbance versus time for all three brands resembled a dissolution profile because as time continued the absorbances increased. When the concentration was plotted versus time, a negative parabola should be plotted on the graph. However this was not observed, instead a positive parabola similar to the average absorbance versus time plots was formed. This trend occurred for all three brands. Figure 14 demonstrates how all three brands follow this trend using Bayer Aleve ® as an example. Appendix 3 contains all of the supplement data for pH 7.4. The positive parabola was formed because these experiments were measuring
<table>
<thead>
<tr>
<th>Product</th>
<th>Average Rate of Dissolution at pH 7.4 (hh:mm:ss)</th>
</tr>
</thead>
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<tr>
<td>Bayer Aleve ®</td>
<td>7:30:52</td>
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<tr>
<td>CVS All Day Pain Relief</td>
<td>2:00:52</td>
</tr>
<tr>
<td>Walgreens All Day Pain Relief</td>
<td>3:30:52</td>
</tr>
</tbody>
</table>

Table 8 – Rate of Dissolution at pH 7.4
Average Percent Dissolved (Bayer Aleve)
Average Percent Dissolved (CVS)

% Dissolved vs Time (hh:mm:ss)
Figure 13 – Average Percent Dissolved at pH 7.4 for Bayer Aleve®, CVS All Day Pain Relief and Walgreens All Day Pain Relief
Figure 14 – Average Absorbance versus Time and Average Concentration versus Time Plots at pH 7.4 for Bayer Aleve®
the product as concentration left the tablet. In other words, the plot was displaying the appearance of the product where as a negative parabola would show the disappearance of the reactant.

A first order kinetic plot was generated for all three brands by plotting the natural log of the concentration versus time in seconds. Figure 15 expresses the average first order kinetic plot for Bayer Aleve ®. The other two brands resembled the same profile and can be found in Appendix 3. Standard deviation was used to determine how the results deviate from the average result. The error bars on the graph seem large however for all three brands the standard deviation was not greater than two. The plot demonstrates the variability between experiments for each brand which was attributed to error caused by the instrument during dissolution. This instrument error could be attributed to background noise, air bubbles on the bottom of the fiber optic probe and/or a short equilibration time. The ReactIR is very sensitive and the slightest disturbance could alter the dissolution testing. The variability was not caused by the tablet itself because the error was so small.

Integrated rate laws typically have a negative slope for the disappearance of the reactant. However, these plots have a positive slope which displays the appearance of the product. The noise level in the beginning of the graph is so high that it is hard to determine what was taking place. Therefore it could not be determined if a first order reaction was observed. This could be the result of an absorbing excipient that was not listed on the label, an unusual release of the drug which could be caused by an unknown binder present in the formula or simply because multiple processes are occurring. Excipients are added to formulas to bulk up formulations and to help guide the active ingredient. Binders hold ingredients in the tablet where as coatings protect the tablets ingredients from moisture.
Figure 15–Average ln[A] versus Time Plot at pH 7.4 for Bayer Aleve ®
At the end of each eight hour period, it was noticed that more powder was present at the bottom of the vessel for the Walgreens All Day Pain Relief tablets versus the other two brands. Another observation during all of the Walgreens experiments was that the time it took for the tablet to lose its coating was a lot longer than the other two brands. These two factors could affect the dissolution and kinetic results for the Walgreens All Day Pain Relief tablets.

Another factor could be the size and shape of the tablet. A larger tablet surface area would cause a different release pattern of the drug. Walgreens All Day Pain Relief is oval in shape unlike Bayer Aleve and CVS All Day Pain Relief which is a round tablet. The reason that Walgreens All Day Pain Relief is oval in shape is because it is a caplet. The formulation of the drug is in tablet form yet it has the look of a capsule. The shape and size of the product is something that formulators decide on when duplicating a formula. This is a good example why Walgreens All Day Pain Relief is a generic of Bayer Aleve. Kinetics suggests what happens during a reaction as well as how fast the reaction is going. In this case the surface area dictates how fast the reaction will go which is dependent on its slope. The oval shape of the caplet takes away concentration kinetics because the concentration of the drug is being released equally over a period of time. Therefore the surface area of the caplet is a function of time and determines the rate at which the drug is delivered. Some examples of this concept are nicotine patches, birth control devices and Wellbutrin XL. All three are independent of concentration and are not immediate release systems. An immediate release of the drug would cause spikes in concentration and have adverse affects on its patients. That reasoning is why these drugs are either extended release or sustained release systems where the concentration of the drug is dispersed evenly over a period of time.
Zero-order kinetics was also evaluated for each brand because the profile seen in Figure 15 was not expected. A zero-order kinetic process demonstrates how concentration is independent of time. The slope of a zero-order kinetics plot is negative which was not observed. The profile was similar to that of the first order kinetic plots. However as the dissolution testing continued the amount of drug was distributed evenly over a period of time. Here as the plot began to level off it formed a straight line which demonstrated zero-order kinetics. Zero-order reactions occur over a short time span because concentration can never be negative.

Towards the end of the dissolution process, the reaction turned into a sustained release system when the concentration of the drug became constant. Once the drug hit maximum concentration, it began to level off into a straight line which can be seen in Figure 16 and Appendix 3. As time evolved, the variation between experiments increased for Bayer Aleve ® yet remained constant throughout for CVS All Day Pain Relief and Walgreens All Day Pain Relief. During a zero-order process, concentration is independent of how much reactant is used. This would make sense as the tablet moves though the body because it is a constant rate process.

3.4 pH 4.5 and pH 1.2 Dissolution Studies

3.4.1 Calibration Difficulties

A blank was taken for 0.05 M phosphate buffer pH 4.5 and simulated gastric fluid pH 1.2. Unlike 0.1 M phosphate buffer pH 7.4, there was not a distinct peak at 1217 cm\(^{-1}\). However there were peaks present to the left and the right of that specific wavenumber. Figure 17 reveals a peak present at 1228 cm\(^{-1}\) and 1194 cm\(^{-1}\) for 0.05 M phosphate buffer
Figure 16–Average Concentration versus Time Plot at pH 7.4 for Bayer Aleve®
and Figure 18 shows a shift in wavenumber to 1220 cm\(^{-1}\) for simulated gastric fluid. All three wavenumbers are still within the C-O functional group range that was chosen to be monitored for the naproxen molecule (1320-1000 cm\(^{-1}\)). A drift in wavenumber could be caused by a fluctuation in temperature if the temperature was not remaining constant and/or the instrument needed to be equilibrated longer before the start of an experiment.

A calibration curve was also generated for 0.05 M phosphate buffer pH 4.5 and simulated gastric fluid pH 1.2. Similar to the previous calibration data, a stock solution (5 mg/mL) and seven dilutions with different concentrations were monitored by the ReactIR. The area under the curve (AUC) was calculated for peaks present in solution that contained naproxen yet absent in the medium. The two plots shown in Figure 19 illustrate the shift in wavenumber (1194 cm\(^{-1}\) and 1228 cm\(^{-1}\)) that occurred at 0.05 M phosphate buffer pH 4.5. The same wavenumber shift occurred when simulated gastric fluid pH 1.2 was used as the medium and is displayed in Figure 20. The plots shown in Figures 19 and 20 do not form a straight line. It seems that as the medium becomes more acidic (lower pH) the naproxen molecule began to protonate which caused the shift in wavenumber. It can be concluded that 0.05 M phosphate buffer is a weak acid at pH 4.5 and simulated gastric fluid is a strong acid at pH 1.2. This trend was not seen in the calibration data for 0.1 M phosphate buffer because pH 7.4 is neutral. This is why the points formed a straight line because no protonation between ions and molecules was taking place.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated in the same manner as mentioned earlier following Equations 9 and 10 to determine the smallest concentration of analyte that can be detected within a sample\(^{35,36}\). These values are listed in Table 9 where the LOQs are larger than the LODs. The line fit plot for the calibration
Figure 17 – Phosphate Buffer pH 4.5
Figure 18 – Simulated Gastric Fluid pH 1.2
Figure 19 – Naproxen Calibration Curve at pH 4.5

Calibration Curve at 1194 cm\(^{-1}\)

\[ y = 0.6122x + 2.0158 \]
\[ R^2 = 0.4945 \]

Calibration Curve at 1228 cm\(^{-1}\)

\[ y = 0.7786x + 3.4522 \]
\[ R^2 = 0.4006 \]
**Figure 20 – Naproxen Calibration Curve at pH 1.2**

- **Calibration Curve at 1194 cm\(^{-1}\)**
  
  \[ y = 0.3605x + 0.0939 \]
  
  \[ R^2 = 0.7323 \]

- **Calibration Curve at 1228 cm\(^{-1}\)**
  
  \[ y = 0.433x + 0.2907 \]
  
  \[ R^2 = 0.5847 \]
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<tr>
<th>Medium</th>
<th>Wavenumber (cm(^{-1}))</th>
<th>Limit of Detection (LOD) Concentration (mg/mL)</th>
<th>Limit of Quantification (LOQ) Concentration (mg/mL)</th>
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<td>0.05 M Phosphate Buffer</td>
<td>1194</td>
<td>3.589</td>
<td>11.963</td>
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<tr>
<td></td>
<td>1228</td>
<td>4.342</td>
<td>14.473</td>
</tr>
<tr>
<td>Simulated Gastric Fluid pH 1.2</td>
<td>1194</td>
<td>2.146</td>
<td>7.154</td>
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<tr>
<td></td>
<td>1228</td>
<td>2.992</td>
<td>9.973</td>
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</table>

Table 9 – Concentration LODs and LOQs at pH 4.5 and pH 1.2
curves can be found on pages 160-167. For both pH levels at the shifted wavenumbers, the concentrations are lower than the LODs which meant that the sample cannot be detected under these conditions because an interference was occurring.

### 3.4.2 Percent Dissolved

Similar to the previous section, dissolution profiles were generated for each brand while using 0.05 M phosphate buffer and simulated gastric fluid as the medium. Each brand dissolved one hundred percent within eight hours however the average dissolution rates differed between the three brands. This trend occurred for each medium and is shown in Tables 10 and 11. Bayer Aleve ® and CVS All Day Pain Relief dissolved the fastest when 0.05 M phosphate buffer was used. For simulated gastric fluid, all three brands dissolved within two hours of the dissolution testing. It was observed that the percent dissolved graphs for these two pH levels were not similar to the percent dissolved graphs presented in the above section when 0.1 M phosphate buffer pH 7.4 was used as the medium. Under pH 1.2, experiments were not reproducible because one experiment seemed to dissolve quicker than the others. Therefore if more experiments were ran, the average rate of dissolution would be different because of the inconsistencies.

Variation among experiments was caused by a drop in baseline during the dissolution testing when using 0.05 M phosphate buffer pH 4.5 as the medium as well as a shift in wavenumber that was observed for pH 4.5 and pH 1.2. These two factors were influenced by the temperature not remaining constant and/or a short instrument equilibration period. Experimental setup may take fifteen to thirty minutes which was followed by a twenty minute equilibration period. With this additional time, the instrument may still need more
<table>
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<tr>
<th>Product</th>
<th>Average Rate of Dissolution at pH 4.5 (hh:mm:ss)</th>
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<tbody>
<tr>
<td>Bayer Aleve ®</td>
<td>2:00:52</td>
</tr>
<tr>
<td>CVS All Day Pain Relief</td>
<td>2:00:52</td>
</tr>
<tr>
<td>Walgreens All Day Pain Relief</td>
<td>4:00:52</td>
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</table>

Table 10 – Rate of Dissolution at pH 4.5
<table>
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<th>Product</th>
<th>Average Rate of Dissolution at pH 1.2 (hh:mm:ss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayer Aleve ®</td>
<td>1:30:52</td>
</tr>
<tr>
<td>CVS All Day Pain Relief</td>
<td>2:00:52</td>
</tr>
<tr>
<td>Walgreens All Day Pain Relief</td>
<td>1:30:52</td>
</tr>
</tbody>
</table>

Table 11 – Rate of Dissolution at pH 1.2
time to warm up before an experiment was started. Another factor could be the temperature probe. The control pad may not have been displaying the correct temperature throughout the entirety of the experiment.

As stated earlier, there was a shift in wavenumber when using 0.05 M phosphate buffer pH 4.5 for the CVS All Day Pain Relief and Walgreens All Day Pain Relief tablets to 1194 cm\(^{-1}\) and 1228 cm\(^{-1}\) respectively. However the wavenumber for Bayer Aleve ® remained at 1217 cm\(^{-1}\). The percent dissolved graphs when using 0.05 M phosphate buffer pH 4.5 resembled a dissolution profile with plenty of error. CVS All Day Pain Relief had the greatest amount of variation throughout the eight hour period which is shown in Figure 21. The plots for Bayer Aleve ® and Walgreens All Day Pain Relief have a large amount of error also which can be found in Appendix 4. It was concluded that because there was so much variation between experiments, as well as a drop in baseline and a shift in wavenumber, this data may not be truly valid.

There was also a shift in wavenumber for CVS All Day Pain Relief and Walgreens All Day Pain Relief tablets, 1194 cm\(^{-1}\) and 1228 cm\(^{-1}\) respectively, when simulated gastric fluid pH 1.2 was used as the medium. However, there was no drop in baseline. The average percent dissolved graphs for CVS All Day Pain Relief and Walgreens All Day Pain Relief do not follow a traditional dissolution profile because the error is so large between experiments, shown in Figure 22. Appendix 5 contains the percent dissolved for Bayer Aleve ® which resembled a dissolution profile when compared to the other two brands. The error in the CVS All Day Pain Relief plot was quite large in the beginning of the experiment but decreased over time. The error observed in all three plots was caused by background noise, a change in temperature of the system and/or a shift in wavenumber.
Figure 21 – Average Percent Dissolved at pH 4.5 for CVS All Day Pain Relief
Figure 22 – Average Percent Dissolved at pH 1.2 for CVS All Day Pain Relief and Walgreens All Day Pain Relief
3.4.3 Kinetic Study

Using the absorbances and Beer Lambert law, the concentrations were calculated using the slope of the line from the appropriate calibration curves. It was noticed that the absorbances and concentrations for Bayer Aleve® and CVS All Day Pain Relief were decreasing with time when 0.05 M phosphate buffer pH 4.5 was used as the medium. This was caused by a variation in temperature which resulted in a drop in baseline throughout the dissolution testing. As time evolved, the absorbances and concentrations decreased as the baseline decreased. This occurrence was not a result of the tablet or the pH level. The average concentration versus time plots produced a negative parabola for Bayer Aleve® and CVS All Day Pain Relief. The negative parabola was a reflectance of the drop in baseline. This meant that the amount of reactant was measured however for dissolution testing the appearance of the product was normally observed. In this case the plot should resemble a positive parabola, which was observed for Walgreens All Day Pain Relief but not Bayer Aleve® and CVS All Day Pain Relief. The positive parabola was comparable to the results produced under pH 7.4 conditions. These differences caused by the drop in baseline are shown in Figure 23 and Appendix 4.

A drop in baseline was not observed when using simulated gastric fluid pH 1.2 as the medium. A positive parabola shown in Figure 24 was produced for all three brands. As previously mentioned, a positive parabola means that as time increased the amount of concentration leaving the tablet was also increasing and the product was formed. Again, these results are comparable to the data produced for pH 7.4 and can be found in Appendix 5. Therefore a drop in baseline can be ruled out as a reason why the percent dissolved experiments were inconsistent when using simulated gastric fluid pH 1.2.
Figure 23 – Average Absorbance versus Time and Average Concentration versus Time Plots at pH 4.5 for CVS All Day Pain Relief
Figure 24 – Average Absorbance versus Time and Average Concentration versus Time Plots at pH 1.2 for Walgreens All Day Pain Relief
The natural log of the concentration was plotted versus time for each brand. Again, variation between experiments was observed for each brand depending on the absorbance and pH level. All of the first order kinetic plots for pH 4.5 resembled a dissolution profile so it could not be determined if a first order reaction occurred. For Bayer Aleve ® and CVS All Day Pain Relief, the plots exhibited a slight decrease in the slope of the line at the beginning of the dissolution testing. This was a consequence of the drop in baseline. The beginning of the dissolution testing for Walgreens All Day Pain Relief displayed a slight increase in the slope of the line which was similar to the appearance of the product. This profile was comparable to what was seen when using 0.1 M phosphate buffer pH 7.4. However, this was not sufficient to conclude if a first order reaction took place. There may be an absorbing excipient or an unknown binder within the formulation that was causing an unusual release of the drug. Other possibilities that could affect the release of the drug are the surface area of the tablet and/or the composition of the solution in which the tablet was dropped. Lastly because multiple processes occurred it caused the absence of a distinct first order process at the beginning of the dissolution testing.

As the reaction continued, the profile began to level off into a straight line. This portion of the plot resembled a zero-order kinetic process because concentration was distributed evenly over time. Again the results for Bayer Aleve ® and CVS All Day Pain Relief were not valid because of the drop in baseline. The behavior of the tablet could not be determined for pH 4.5 because the drop in baseline caused the absorbances to decrease. In the plots below, the error bars seem quite significant because the scale of the y-axis is fairly small. The standard deviation was not greater than two for the three brands and is
demonstrated in Figures 25 and 26. The average first order kinetic plots and the average zero-order kinetic plots for the other two brands can be found in Appendix 4.

Similar results were observed for simulated gastric fluid pH 1.2. When the natural log of the concentration was plotted versus time, the points began to level off forming a straight line as time evolved. This was an example of a constant rate process. Figure 27 displays the average first order kinetic plot which confirmed that it could not be determined if a first order reaction took place. The slightly positive slope in the beginning of the dissolution testing was an example of the appearance of the product rather than the elimination of the reactant. However, this was not enough evidence that a distinct first order reaction was occurring. Similarly to the other two pH levels, this notion that two processes are occurring throughout the dissolution process was the reason why a clear first order reaction was not observed. The error bars are very small between all three brands and the plots are very similar. For the average zero-order kinetics plot, Figure 28, the error bars again seem rather large because the scale of the y-axis is so small and not a result of a large variation between experiments. The average first order kinetic plots and the average zero-order kinetic plots for the other two brands can be found in Appendix 5.
Figure 25–Average ln[A] versus Time Plot at pH 4.5 for CVS All Day Pain Relief
Figure 26–Average Concentration versus Time Plot at pH 4.5 for CVS All Day Pain Relief
Figure 27–Average ln[A] versus Time Plot at pH 1.2 for Walgreens All Day Pain Relief
Figure 28–Average Concentration versus time plot at pH 1.2 for Walgreens All Day Pain Relief
3.5 Linear Regression Models

A regression model was constructed for Bayer Aleve®, CVS All Day Pain Relief and Walgreens All Day Pain Relief under each pH level. A linear regression demonstrates the relationship between experimental values and predicted values. Residual analysis is the difference between the observed value (y) and the predicted value (ŷ). This means that a residual plot will determine the quality of regression by expressing if the observed error (the residuals) is consistent with random error. In other words, the residuals are plotted as a function of the independent variability. A residual plot can be created by plotting the residuals (e) versus the independent variable (X).  

There are two kinds of error, random and non-random. Random errors will produce a residual plot that has normal distribution of points found in a symmetrical pattern. This symmetrical pattern consists of errors that are evenly distributed above and below the x-axis. Random error causes a different result each time a measurement is repeated under the same conditions. If this occurs then the data presented is a good fit and a linear regression model can be used. If the distribution is non-random then the points will form a U-shaped curve pattern or an inverted-U pattern. If this pattern is observed then a non-linear regression model is the best fit for the data.  

Analysis of variance (ANOVA) separates and estimates differences that are caused by variation. This randomness is an important factor for a regression model and will help determine if a linear or non-linear model should be used on the data. Line fit plots and residual plots were created to analyze the calibration curves at each pH level, as well as the average first order kinetic plots and the average zero-order kinetic plots for each brand at all three pH levels. It was observed that if the line fit plot has a positive slope, the residual plot
Figure 29 – Linear Regression Residual Analysis
will either have symmetrical points scattered above and below the x-axis or form an inverted-U pattern. On the other hand if the line fit plot has a negative slope, the residual plot will form a U-shaped curve pattern. If the residual plot forms a U-shaped curve pattern or an inverted-U pattern then the points are non-random and a non-linear regression model should be used on the data. In this case it is important to question the data and contemplate if something else was happening during the transition of reactant to product. Naproxen did not change as the concentration was being distributed from the tablet so the reactant and product were the same over the eight hour dissolution period. There may be some sort of interference that was occurring during the dissolution process. Perhaps over the eight hours, multiple processes were occurring as the tablet dissolved. This process could be the transition of an immediate release system to a sustained release system. This could be the reason why a distinct first order reaction was not observed for each brand which would cause a non-linear regression model to be produced.

3.5.1 Line Fit Plot and Residual Plot for Calibration Curve

The line fit plot for all three pH levels produced a positive slope. As a result the residual plots formed a symmetrical pattern above and below the x-axis. This is shown in Figure 30 as well as Appendix 6. A symmetrical pattern means that the data is a good fit and the error is random. This makes sense because the naproxen standard which was used for the calibration curves is a pure substance.
Figure 30 – Line Fit Plot and Residual Plot for pH 7.4 Calibration Curve
3.5.2 pH 7.4 Dissolution Study

The line fit plot for all three brands produced a positive slope which formed an inverted-U pattern on the residual plot. The line fit plot and residual plot of the first order kinetics and zero-order kinetics for Bayer Aleve ® are shown in Figures 31 and 32. First order and zero-order plots for CVS All Day Pain Relief and Walgreens All Day Pain Relief can be found in Appendix 7. A U-shaped curve pattern and an inverted-U pattern on each residual plot expressed that the data was not a good fit and the error was non-random. This makes sense because there are two processes occurring during the dissolution testing as well as a possible interference in the formulation of the drug.

3.5.3 pH 4.5 Dissolution Study

All three brands under pH 4.5 conditions produced line fit plots with either a positive or negative slope. Therefore the residual plots formed a U-shaped curve pattern or an inverted-U pattern. The line fit plot and residual plot of the first order kinetics and zero-order kinetics for CVS All Day Pain Relief are shown in Figures 33 and 34. First order and zero-order plots for Bayer Aleve ® and Walgreens All Day Pain Relief can be found in Appendix 8. Again a U-shaped curve pattern and an inverted-U pattern on each residual plot explained that the data was not a good fit and the error was non-random. The error was non-random because of the transition between an immediate release system to a sustained release system or possibility due to an intermediate between the transitions.
Figure 3.1 – First Order Kinetics: Line Fit and Residual Plot for Bayer Aleve® at pH 7.4
Figure 32 – Zero-order Kinetics: Line Fit and Residual Plot for Bayer Aleve® at pH 7.4
Figure 33 – First Order Kinetics: Line Fit and Residual Plot for CVS All Day Pain Relief at pH 4.5
Figure 3 – Zero-order Kinetics: Line Fit and Residual Plot for CVS All Day Pain Relief at pH 4.5
3.5.4 pH 1.2 Dissolution Study

Similar to the results found in the pH 7.4 dissolution study, all experiments under pH 1.2 produced line fit plots with a positive slope for all three brands. Therefore the residual plots formed an inverted-U pattern. The line fit plot and residual plot of the first order kinetics and zero-order kinetics for Walgreens All Day Pain Relief are shown in Figures 35 and 36. First order and zero-order plots for Bayer Aleve® and CVS All Day Pain Relief can be found in Appendix 9. An inverted-U pattern on the residual plot expressed that the data was not a good fit and the error was non-random. The error was non-random because a multistep process was occurring when the system transitioned from an immediate release to a sustained release.
Figure 35 – First Order Kinetics: Line Fit and Residual Plot for Walgreens All Day Pain Relief at pH 1.2
Figure 36 – Zero-order Kinetics: Line Fit and Residual Plot for Walgreens All Day Pain Relief at pH 1.2
4. Conclusions

It was confirmed that the ReactIR is a very sensitive instrument and different than traditional dissolution. There was plenty of error that occurred when using the ReactIR and EasyMax workstation which was demonstrated throughout many of the experiments. Some examples of error observed dealt with the change in temperature, air bubbles on the bottom of the fiber optic probe, the placement of the tablet in the vessel, the alignment of the probe and instrument, and background noise. These examples would cause variation between experiments. The preparation of the buffer solutions/simulated gastric fluid and the standard as well as a change in pH would also affect the dissolution rate of the tablet.

Besides the differences in the rate of dissolution, the greatest variance was found at the beginning of every dissolution experiment. This could be attributed to either the instrument needed a longer equilibration time and/or the temperature not remaining constant. All three brands followed the same trend as the tablet dissolved; the drug was released at a fast rate and leveled off after a period of time. Table 12 confirms that Bayer Aleve ®, CVS All Day Pain Relief and Walgreens All Day Pain Relief tablets dissolved one hundred percent in an eight hour period at pH 7.4, pH 4.5 and pH 1.2. Therefore, it was concluded that the rate of dissolution was comparable to what was stated on each brands corresponding label: take one tablet every 8 hours.

A drop in baseline affected the kinetic studies for Bayer Aleve ® and CVS All Day Pain Relief when using 0.05 M phosphate buffer pH 4.5. Also a shift in wavenumber affected CVS All Day Pain Relief and Walgreens All Day Pain Relief when subjected to 0.05 M phosphate buffer pH 4.5 and simulated gastric fluid pH 1.2. There was no drop in baseline and no shift in wavenumber for any brand while using 0.1 M phosphate buffer pH 7.4. The shift in wavenumber could be attributed to fluctuating temperature during the dissolution
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<th>Average Rate of Dissolution at pH 4.5 (hh:mm:ss)</th>
<th>Average Rate of Dissolution at pH 1.2 (hh:mm:ss)</th>
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</thead>
<tbody>
<tr>
<td>Bayer Aleve ®</td>
<td>7:30:52</td>
<td>2:00:52</td>
<td>1:30:52</td>
</tr>
<tr>
<td>CVS All Day Pain Relief</td>
<td>2:00:52</td>
<td>2:00:52</td>
<td>2:00:52</td>
</tr>
<tr>
<td>Walgreens All Day Pain Relief</td>
<td>3:30:52</td>
<td>4:00:52</td>
<td>1:30:52</td>
</tr>
</tbody>
</table>

Table 12 – Comparison of Dissolution Rates between Bayer Aleve®, CVS All Day Pain Relief and Walgreens All Day Pain Relief
testing, yet it seemed as conditions became more acidic the wavenumber began to shift either to the left or the right of the initial wavenumber. This shift could be caused by the protonation of naproxen as conditions became more acidic. Another possibility could be that the shift was a result of the sodium salt component of naproxen as it interacted with a different environment. Overall, if there was no drop in baseline and no shifting wavenumber at pH 4.5 and pH 1.2, each brand would still dissolve one hundred percent in an eight hour period.

It was assumed that Naproxen would follow a first order kinetic process because immediate release systems tend to follow that trend. However the points did not form a distinct negative slope. All of the first order plots do show a slight increase in the beginning of the dissolution experiments which resembled the appearance of the product. It was concluded that there could be an absorbing excipient not listed on the label, an unknown binder within the tablets formulation, an intermediate step occurring as the reactant became the product and/or multiple processes occurring throughout the dissolution testing because the slope was not as prominent as imagined. As a result, it could not be concluded if a first order kinetics reaction occurred at the beginning of the dissolution profile. The transition between an immediate release system to a sustained release system may be the reason why there was no distinct positive slope that would distinguish first order kinetics. Towards the end of the kinetic profile, the points began to level off and formed a straight line. Typically a flat line means that the system has become independent of the concentration therefore zero-order kinetics applies.
5. Literature Cited

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14. USP. <711> Dissolution


16. Khan Academy. Thermodynamics


21. Controlling Drug Delivery


26. USP. *US Pharmacopeia National Formulary*; Reagents, Indicators, Solutions; Vol. USP 36, p 8

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http://depts.washington.edu/cpac/Activities/Meetings/Summer/2009/Wednesday/Scholl%


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Appendix 1: Packaging and Formulation

6.1 Procedure for Operating the EasyMax and ReactIR

*Position Probe*

*Align Probe*
6.1 Procedure for Operating the EasyMax and ReactIR

Clean Probe

Collect Background
6.1 Procedure for Operating the EasyMax and ReactIR

Collect Reference Samples

Spectra Library
6.1 Procedure for Operating the EasyMax and ReactIR

**Start screen**

**Experiment started**
Appendix 2: GC-MS Data

2.1 Sum of Corrected Areas (GC-MS) for Extracted S-Naproxen from Bayer Aleve ®
(Advanced Biotech)

<table>
<thead>
<tr>
<th>Peak</th>
<th>R.T. first</th>
<th>Scan min</th>
<th>Scan max</th>
<th>Scan last</th>
<th>PK</th>
<th>Height</th>
<th>Corr. area</th>
<th>% max</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.145</td>
<td>815</td>
<td>820</td>
<td>824</td>
<td>M2</td>
<td>16059</td>
<td>204473</td>
<td>1.17%</td>
<td>1.139%</td>
</tr>
<tr>
<td>2</td>
<td>16.253</td>
<td>3636</td>
<td>3648</td>
<td>3692</td>
<td>M5</td>
<td>3043</td>
<td>182378</td>
<td>1.04%</td>
<td>1.016%</td>
</tr>
<tr>
<td>3</td>
<td>19.892</td>
<td>4483</td>
<td>4498</td>
<td>4516</td>
<td>M3</td>
<td>4830</td>
<td>105115</td>
<td>0.60%</td>
<td>0.586%</td>
</tr>
<tr>
<td>4</td>
<td>20.744</td>
<td>4661</td>
<td>4697</td>
<td>5134</td>
<td>M</td>
<td>202788</td>
<td>17456163</td>
<td>100.00%</td>
<td>97.259%</td>
</tr>
</tbody>
</table>

Sum of corrected areas: 17948128

<table>
<thead>
<tr>
<th>Peak</th>
<th>R.T. Start</th>
<th>End</th>
<th>Scan min</th>
<th>Scan max</th>
<th>Scan last</th>
<th>PK</th>
<th>Height</th>
<th>Corr. area</th>
<th>% max</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.146</td>
<td>M</td>
<td>4.128</td>
<td>4.161</td>
<td>M</td>
<td>5165</td>
<td>58781</td>
<td>0.17%</td>
<td>0.166%</td>
<td>Ethyl Acetate</td>
</tr>
<tr>
<td>2</td>
<td>16.245</td>
<td>16</td>
<td>16.197</td>
<td>16.627</td>
<td>M</td>
<td>5811</td>
<td>348260</td>
<td>1.00%</td>
<td>0.984%</td>
<td>4-Methoxybiphenyl</td>
</tr>
<tr>
<td>3</td>
<td>19.885</td>
<td>19</td>
<td>19.850</td>
<td>19.981</td>
<td>M</td>
<td>7253</td>
<td>169698</td>
<td>0.49%</td>
<td>0.479%</td>
<td>Methyl Naproxen</td>
</tr>
<tr>
<td>4</td>
<td>20.736</td>
<td>20</td>
<td>20.321</td>
<td>23.430</td>
<td>M</td>
<td>479233</td>
<td>34814735</td>
<td>100.00%</td>
<td>98.370%</td>
<td>Naproxen</td>
</tr>
</tbody>
</table>
Appendix 2: GC-MS Data

2.2 Sum of Corrected Areas (GC-MS) for Extracted S-Naproxen from CVS All Day Pain Relief (Advanced Biotech)

Area Percent Report

Data Path : C:\DATA\1405\ 
Data File : EXPCVS000.D
Acq On : 20 May 2014 10:14 (#1); 20 May 2014 10:13 (#2)
Operator : PAL
Sample : CVS NAPROXEN
Misc : ZB100ND_F, SP50:1, IN METHANOL (Sig #1); (Sig #2)
ALS Vial : 1 Sample Multiplier: 1

Integration Parameters: autoint.e
Integrator: ChemStation 6890 Scale Mode: Large solvent peaks clipped
Method : C:\msdchem\1\5973H\ZB100ND_F.M

Signal : TIC: EXPCVS000.D\data.ms

<table>
<thead>
<tr>
<th>peak</th>
<th>R.T.</th>
<th>first</th>
<th>max</th>
<th>last</th>
<th>PK</th>
<th>peak</th>
<th>corr.</th>
<th>corr.</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>min</td>
<td>scan</td>
<td>scan</td>
<td>scan</td>
<td>scan</td>
<td>height</td>
<td>area</td>
<td>% max.</td>
<td>total</td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>--------</td>
<td>-------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>1</td>
<td>4.144</td>
<td>814</td>
<td>820</td>
<td>836</td>
<td>M</td>
<td>146904</td>
<td>2561296</td>
<td>11.46%</td>
<td>10.285%</td>
</tr>
<tr>
<td>2</td>
<td>20.776</td>
<td>4636</td>
<td>4704</td>
<td>5135</td>
<td>M</td>
<td>438261</td>
<td>22342887</td>
<td>100.00%</td>
<td>89.715%</td>
</tr>
</tbody>
</table>

Sum of corrected areas: 24904183

Sigal : EXPCVS000.D\PID1A.CH

<table>
<thead>
<tr>
<th>peak</th>
<th>R.T.</th>
<th>Start</th>
<th>End</th>
<th>PK</th>
<th>peak</th>
<th>corr.</th>
<th>corr.</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>min</td>
<td>min</td>
<td>min</td>
<td>min</td>
<td>min</td>
<td>height</td>
<td>area</td>
<td>% max.</td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>--------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>1</td>
<td>4.145</td>
<td>4.120</td>
<td>4.178</td>
<td>M</td>
<td>64166</td>
<td>998545</td>
<td>2.18%</td>
<td>2.136% Ethyl Acetate</td>
</tr>
<tr>
<td>2</td>
<td>20.771</td>
<td>19.986</td>
<td>23.810</td>
<td>M</td>
<td>768411</td>
<td>45745545</td>
<td>100.00%</td>
<td>97.864% Naproxen</td>
</tr>
</tbody>
</table>

Sum of corrected areas: 46744090

ZB100ND_F.M Wed Jan 21 10:31:45 2015
Appendix 2: GC-MS Data

2.3 Sum of Corrected Areas (GC-MS) for Extracted S-Naproxen from Walgreens All Day Pain Relief (Advanced Biotech)

Area Percent Report

Data Path: C:\DATA\1405\  
Data File: EXPCVS002.D  
Acq On: 20 May 2014 10:57 (#1); 20 May 2014 10:56 (#2)  
Operator: PAL  
Sample: WALGREENS NAPROXEN  
Misc: 2B100ND_F, SP50:1, IN METHANOL (Sig #1); (Sig #2)  
ALS Vial: 2 Sample Multiplier: 1  
Integration Parameters: autoint1.e  
Integrator: ChemStation 6890 Scale Mode: Large solvent peaks clipped  
Method: C:\msdchem\1\5973N\2B100ND_F.M

<table>
<thead>
<tr>
<th>peak</th>
<th>R.T</th>
<th>first</th>
<th>max</th>
<th>last</th>
<th>PK</th>
<th>peak corr.</th>
<th>corr.</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.239</td>
<td>3636</td>
<td>3645</td>
<td>3690</td>
<td>M5</td>
<td>9373</td>
<td>402191</td>
<td>0.68%</td>
</tr>
<tr>
<td>2</td>
<td>19.888</td>
<td>4485</td>
<td>4497</td>
<td>4512</td>
<td>M2</td>
<td>9332</td>
<td>191044</td>
<td>0.32%</td>
</tr>
<tr>
<td>3</td>
<td>20.797</td>
<td>4665</td>
<td>4709</td>
<td>5157</td>
<td>M</td>
<td>1068858</td>
<td>59259654</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Sum of corrected areas: 59852889

<table>
<thead>
<tr>
<th>peak</th>
<th>R.T</th>
<th>Start</th>
<th>End</th>
<th>PK</th>
<th>peak corr.</th>
<th>corr.</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.145</td>
<td>4.129</td>
<td>4.167</td>
<td>M</td>
<td>10158</td>
<td>119952</td>
<td>0.16% 0.153% Ethyl Acetate</td>
</tr>
<tr>
<td>2</td>
<td>16.235</td>
<td>16.196</td>
<td>16.536</td>
<td>M</td>
<td>15817</td>
<td>731744</td>
<td>0.95% 0.933% 4-Methoxy biphenyl</td>
</tr>
<tr>
<td>3</td>
<td>19.883</td>
<td>19.847</td>
<td>19.969</td>
<td>M</td>
<td>13309</td>
<td>273174</td>
<td>0.35% 0.348% Methyl Naproxen</td>
</tr>
<tr>
<td>4</td>
<td>20.792</td>
<td>20.241</td>
<td>22.906</td>
<td>M</td>
<td>1127423</td>
<td>71323668</td>
<td>100.00% 98.566% Naproxen</td>
</tr>
</tbody>
</table>
Appendix 3: pH 7.4 Supplement Data

3.1 Average Absorbance versus Time and Average Concentration versus Time Plots at pH 7.4
3.1 Average Absorbance versus Time and Average Concentration versus Time Plots at pH 7.4

![Average Absorbance vs Time (Walgreens)](image)

![Average Concentration vs Time (Walgreens)](image)
Appendix 3: pH 7.4 Supplement Data

3.2 Average First Order Kinetics Plot at pH 7.4
3.2 Average First Order Kinetics Plot at pH 7.4
Appendix 3: pH 7.4 Supplement Data

3.3 Average Zero-order Kinetics Plot at pH 7.4

![Average Zero Order Kinetics (CVS)](image)
3.3 Average Zero-order Kinetics Plot at pH 7.4
Appendix 4: pH 4.5 Supplement Data

4.1 Average Percent Dissolved at pH 4.5

![Graph of Average Percent Dissolved (Bayer Aleve)](image)

- Percentage dissolved on the y-axis.
- Time (hh:mm:ss) on the x-axis.

The graph shows the average percent dissolved over time for Bayer Aleve at pH 4.5.
4.1 Average Percent Dissolved at pH 4.5
Appendix 4: pH 4.5 Supplement Data

4.2 Average Absorbance versus Time and Average Concentration versus Time Plots at pH 4.5
4.2 Average Absorbance versus Time and Average Concentration versus Time Plots at pH 4.5
Appendix 4: pH 4.5 Supplement Data

4.3 Average First Order Kinetics Plot at pH 4.5
4.3 Average First Order Kinetics Plot at pH 4.5
Appendix 4: pH 4.5 Supplement Data

4.4 Average Zero-order Kinetics Plot at pH 4.5

![Average Zero-Order Plot (Bayer Aleve)](image-url)
4.4 Average Zero-order Kinetics Plot at pH 4.5
Appendix 5: pH 1.2 Supplement Data

5.1 Average Percent Dissolved at pH 1.2

![Graph showing average percent dissolved at pH 1.2](image-url)
Appendix 5: pH 1.2 Supplement Data

5.2 Average Absorbance versus Time and Average Concentration versus Time Plots at pH 1.2
5.2 Average Absorbance versus Time and Average Concentration versus Time Plots at pH 1.2

![Average Absorbance vs Time (CVS)](image1)

![Average Concentration vs Time (CVS)](image2)
Appendix 5: pH 1.2 Supplement Data

5.3 Average First Order Kinetic Plot at pH 1.2

![Average First Order Kinetics (Bayer Aleve)](image-url)
5.3 Average First Order Kinetic Plot at pH 1.2
Appendix 5: pH 1.2 Supplement Data

5.4 Average Zero-order Kinetic Plot at pH 1.2
5.4 Average Zero-order Kinetic Plot at pH 1.2
Appendix 6: Line Fit Plot and Residual Plot for Calibration Curve

6.1 Line Fit Plot and Residual Plot for pH 4.5 Calibration Curve (1194 cm$^{-1}$)
6.1 Line Fit Plot and Residual Plot for pH 4.5 Calibration Curve (1194 cm$^{-1}$)
Appendix 6: Line Fit Plot and Residual Plot for Calibration Curve

6.2 Line Fit Plot and Residual Plot for pH 4.5 Calibration Curve (1228 cm\(^{-1}\))
6.2 Line Fit Plot and Residual Plot for pH 4.5 Calibration Curve (1228 cm\(^{-1}\))
Appendix 6: Line Fit Plot and Residual Plot for Calibration Curve

6.3 Line Fit Plot and Residual Plot for pH 1.2 Calibration Curve (1194 cm\(^{-1}\))
6.3 Line Fit Plot and Residual Plot for pH 1.2 Calibration Curve (1194 cm$^{-1}$)
Appendix 6: Line Fit Plot and Residual Plot for Calibration Curve

6.4 Line Fit Plot and Residual Plot for pH 1.2 Calibration Curve (1228 cm$^{-1}$)
6.4 Line Fit Plot and Residual Plot for pH 1.2 Calibration Curve (1228 cm$^{-1}$)
Appendix 7: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 7.4

7.1 First Order Kinetics: Line Fit and Residual Plot for CVS All Day Pain Relief
7.1 First Order Kinetics: Line Fit and Residual Plot for CVS All Day Pain Relief
Appendix 7: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 7.4

7.2 First Order Kinetics: Line Fit and Residual Plot for Walgreens All Day Pain Relief
7.2 First Order Kinetics: Line Fit and Residual Plot for Walgreens All Day Pain Relief
Appendix 7: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 7.4

7.3 Zero-order Kinetics: Line Fit and Residual Plot for CVS All Day Pain Relief
7.3 Zero-order Kinetics: Line Fit and Residual Plot for CVS All Day Pain Relief
Appendix 7: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 7.4

7.4 Zero-order Kinetics: Line Fit and Residual Plot for Walgreens All Day Pain Relief
7.4 Zero-order Kinetics: Line Fit and Residual Plot for Walgreens All Day Pain Relief
Appendix 8: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 4.5

8.1 First Order Kinetics: Line Fit and Residual Plot for Bayer Aleve ®
8.1 First Order Kinetics: Line Fit and Residual Plot for Bayer Aleve®
Appendix 8: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 4.5

8.2 First Order Kinetics: Line Fit and Residual Plot for Walgreens All Day Pain Relief
8.2 First Order Kinetics: Line Fit and Residual Plot for Walgreens All Day Pain Relief
Appendix 8: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 4.5

8.3 Zero-order Kinetics: Line Fit and Residual Plot for Bayer Aleve ®
8.3 Zero-order Kinetics: Line Fit and Residual Plot for Bayer Aleve®
Appendix 8: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 4.5

8.4 Zero-order Kinetics: Line Fit and Residual Plot for Walgreens All Day Pain Relief
8.4 Zero-order Kinetics: Line Fit and Residual Plot for Walgreens All Day Pain Relief
Appendix 9: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 1.2

9.1 First Order Kinetics: Line Fit and Residual Plot for Bayer Aleve ®
9.1 First Order Kinetics: Line Fit and Residual Plot for Bayer Aleve®
Appendix 9: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 1.2

9.2 First Order Kinetics: Line Fit and Residual Plot for CVS All Day Pain Relief
9.2 First Order Kinetics: Line Fit and Residual Plot for CVS All Day Pain Relief
Appendix 9: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 1.2

9.3 Zero-order Kinetics: Line Fit and Residual Plot for Bayer Aleve ®
9.3 Zero-order Kinetics: Line Fit and Residual Plot for Bayer Aleve ®
Appendix 9: Line Fit Plot and Residual Plot for First Order and Zero-order Kinetics pH 1.2

9.4 Zero-order Kinetics: Line Fit and Residual Plot for CVS All Day Pain Relief
9.4 Zero-order Kinetics: Line Fit and Residual Plot for CVS All Day Pain Relief