



**NATURAL
SCIENCES**

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THE EFFECTS OF PH ON THE ABSORPTION OF FORTIFIED IRON

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Abstract

Have you ever experienced a burning pain on your sternum before? This burning is caused by gastroesophageal reflux. This is a common disease where the acid from the stomach travels back up the esophagus. The only way to contain this burning is to take a gastroesophageal reflux medication. These medications are taken to decrease the acidity of the stomach acid.

These medications could also cause a new problem called iron-deficient anemia, because iron gets less bioavailable as the pH of the stomach rises over 3.5. This investigation was designed to address the question, how does Gastroesophageal reflux medication affect the pH in the stomach, therefore, affecting the bioavailability of iron? I hypothesized that as the pH of the stomach becomes less acidic, because of the effects of the gastroesophageal reflux medication, the bioavailability of iron will decrease.

For this project, gastric acids from the pH of 1 to the pH of 7 were individually heated to 37°C, the average human body temperature. That temperature was maintained for 3 hours in order to mimic the amount of time food would be in the stomach. Ten grams of iron powder were added to solutions at each pH level for 3 hours. After 3 hours, the gastric juice was filtered. The filter paper and iron residue were allowed to dry and then the mass was measured. The amount of iron which was dissolved in the solution was then determined. .

Overall, my hypothesis was incorrect because the bioavailability

of iron was variable at various pH levels. There was not a linear relationship between iron solubility and pH. These results are relevant to this issue because it has been determined that the solubility of fortified iron in the stomach is relatively unchanged by pH variations due to the effect of acid reflux medicine.

Key words: *Acid Reflux, Iron Fortification, pH, Bioavailability, Gastroesophageal reflux medication, iron*

Introduction

Have you ever experienced a burning pain on your sternum before? This burning is caused by gastroesophageal reflux better known as Acid Reflux. Acid Reflux is a common disease where the acid from the stomach travels back up the esophagus. 20% of adults have GERD which is the serious version of acid reflux. The only way to contain this burning is to take a gastroesophageal reflux medication. There are three types of medication that range from minor to serious which is a GERD medication. These medications are taken to decrease the acidity of the stomach, but what other problems could that decrease in acidity be causing?

I was drawn to this project because I experience severe acid reflux to where I have been in the hospital on two different occasions for it. I started on a daily medication, called famotidine, but I realized that every time I stood up, I would get very dizzy. I also noticed that I was constantly feeling tired to where I couldn't move my arms or my legs. I decided to stop the medication and my feelings of fatigue and dizziness went away. This has drawn me to explore how the medications that are used to help diminish acid reflux are affecting the stomach other than the acidity level.

Purpose

Struggling with Acid Reflux/Gerd has been one of the hardest things I have had to do as a person. This digestive disease has made me have to alter my diet completely, and it has been a challenge. Not only have there been multiple hospital visits but there have also been multiple different medications that I have been put on to find which one works best for me. The consistent problem with these medication attempts is a decrease in my ability to breathe, dizziness, and frequent passing out.

Question

The normal pH of the stomach is very acidic, ranging from 1.5 to 3.5. For someone experiencing gastroesophageal reflux and taking medicine for it, the pH of the stomach is above 4. Iron is soluble at the low pH of the stomach, but is insoluble at any pH above 3.5. How does Gastroesophageal reflux medication affect the pH in the stomach, therefore, affecting the bioavailability of iron?

Hypothesis

As the pH of the stomach becomes less acidic, the gastroesophageal reflux medication will decrease the acidity of the stomach even more which will decrease the bioavailability of iron.

Goal

The goal of this experiment is to determine at which pH level iron's bioavailability decreases, which replicates the decrease in acidity by consuming gastroesophageal reflux medication.

Research

Approximately 20% of people in the United States have GERD (gastroesophageal reflux disease) or Acid reflux, which is the lesser version of GERD ("Definition & Facts for GER & GERD"). Acid reflux

happens when the lower esophageal sphincter doesn't close as soon as food passes. This food takes 6-8 hours to pass completely through the stomach, which makes it more likely for acid reflux to occur throughout those 6-8 hours. This creates a way for acid produced by the stomach to travel up the esophagus (Stuart). The most common symptoms of Acid reflux are heartburn and regurgitation. Heartburn is a burning sensation in the chest behind the breastbone. Regurgitation is the feeling of fluid or food coming up into the chest ("Acid Reflux"). There are only three types of treatments that a person with GERD can take, antacids, H₂-receptors, and proton pump inhibitors. All three treatments reduce the acidity of the stomach. 80% of those with GERD have Iron Deficiency Anemia (Stein, 2016). Iron is naturally present in foods and can artificially be included in foods. There are two types of iron found in foods, heme and nonheme. Heme iron is only found in meat, fish, and poultry. Heme iron is absorbed up to 30%. Eating heme iron increases iron levels more than non-heme iron does. Non Heme iron is found in plant foods and iron-fortified food products. These fortified food products create a defensive wall that is built to strengthen against attack.

Iron is an essential component of hemoglobin, which is an erythrocyte protein that transfers oxygen from the lungs to the tissues ("Office of Dietary Supplements - Iron"). 66% of the body's iron intake is used in hemoglobin. Iron is also a participant in the functions of immunity, cell division and differentiation, energy metabolism. Iron homeostasis is controlled by intestinal absorption which makes iron bioavailability detrimental in order to not have iron deficiency anemia (Piskin, 2022). Iron enters the stomach from the esophagus where it is oxidized into the Iron (III) state no matter what its original form was.

There are two types of irons when it comes to reactions. There are ferrous iron and ferric iron. Ferrous iron has a charge of 2+ while Ferric iron has a charge of 3+. Ferrous Iron is completely dissolved no matter what the pH is. Ferric iron, however, will only dissolve in a pH

that is less than 3.5. The stomach has a pH ranging from 1.5 to 3.5. When iron is in this pH, the iron will precipitate. If this iron is in a pH that is larger than 3.5, the iron will become insoluble and precipitate into an orange/yellow compound called yellow boy ("AMD Basics"). Whether the iron is soluble or insoluble at a determined pH value, will determine whether the iron is bioavailable.

The bioavailability of iron ranges from 14% to 18% from a mixed diet and 5% to 12% from vegetarian diets, which all depend on the consumption of heme or nonheme iron. Heme iron has a higher bioavailability than non-heme iron, meaning when heme iron is introduced into the circulation of the body, more heme iron will create an effect in the body than non-heme iron ("Office of Dietary Supplements - Iron"). Dietary inhibitors such as Calcium, Phytates, Polyphenols, and enhancers such as ascorbic acid and proteins are the main influencers on the determination of iron bioavailability. Also, iron bioavailability is determined by the food consumed. Iron bioavailability can also be affected by the individual's iron status, and the individual relying upon factors such as obesity, sicknesses, and other things. The absorption rate of iron has a rate of 25%-30% in the consumption of organ meats, 7%-9% in green leafy vegetables, and 4% in grains (Piskin, 2022). These percentages are affected by Gastroesophageal reflux medication.

As previously said there are three medications for acid reflux, antacids, Histamine-2 (H-2) Receptors, and Proton Pump Inhibitors. Antacids and H-2 receptors are used for infrequent heartburn, while if it is a more frequent occurrence it is best to use a high dose of an H-2 Receptor or a Proton Pump Inhibitor. Antacids are usually taken when one knows the symptoms of their heartburn. H-2 Receptors are taken for more severe acid reflux and they work by blocking the chemical receptors in the stomach called histamine-2 Receptors. This medication lowers the amount of acid that's made in the stomach. These medications are not for long periods of time because the stomach

adapts to the medication over time and the medication becomes less effective. Proton Pump Inhibitors are for those with GERD. These Proton Pump Inhibitors take action by blocking the enzyme that creates stomach acid. The Proton Pump Inhibitors can do this because they actually work in the cells of the stomach lining (Gragnotati, 2022). These medications cause Iron deficiency Anemia.

The people in the world who have acid reflux or GERD also have iron deficiency anemia. This is because of the decrease in acidity in the stomach. The pH of the stomach ranges from 1.5 to 3.5 and iron is only soluble in a pH under 3.5. As people take acid reflux medicine, the point is to decrease the acidity of the stomach, but this also causes an increase in the pH, which then causes a change in the bioavailability of iron. This leads me to hypothesize that as the pH of the stomach becomes less acidic, the gastroesophageal reflux medication will decrease the acidity of the stomach even more which will decrease the bioavailability of iron. Knowing the results will help people to be directed to the correct medication and dosage of that medication to help their acid reflux or GERD, while refraining from having iron deficiency anemia.

Materials

Hydrochloric acid pH 1-8	Quantitative Filter Paper
Iron Powder	Funnel
Funnel Stand	Quantitative Balance
Lab Glassware	Personal Protective Equipment
Hotplate	Waterproof Temperature Sensor
Computer	Small Container

Procedures

1. Prepare hydrochloric acid of various pH (1-8). Remember to wear safety gear.
 - a. To prepare the hydrochloric acid to appropriately

accommodate each individual pH level, find the molarity of each pH level starting with 1. Find this by setting the $[H^+] = \text{antilog}^{-pH}$ value being found.

- b. After finding the molarity for each pH value, find the correct amount of both acid and water for the solution. Find this by using the equation $M_1V_1 = M_2V_2$. The answer will be in liters which will need to be converted to mL.
 - c. Last, combine the acid and the water by adding the water to the acid.
2. Heat to 37°C using a hotplate
 3. Weigh the plastic container used to hold the iron powder
 4. Weigh 10 grams of elemental iron using a quantitative balance
 5. Add 10 grams of elemental iron to each of the samples of hydrochloric acid at the various pH's
 6. Frequently stir the solutions to mimic the stomach environment
 7. Wait 3 and a half hours of maintained heat to drain
 8. Measure the mass of each of 8 pieces of filter paper set up for each specific pH level
 9. Run each solution through a funnel with a single filter paper to collect the undissolved iron.
 10. Let the filter paper and iron dry and record the mass that was weighed.
 11. Add up the weight of the filter paper and the plastic container from the beginning. Take the mass from the iron that is dried and subtract the total mass of the filter paper and the plastic container.
 12. This number should either be less than 10 g meaning that iron dissolved, which means it is bioavailable, or it will be the same 10g meaning that no iron dissolved.
 13. Record all the masses in a notebook or on a computer.

Results and Analysis

pH	Trial 1 Dissolved Iron (g)	Trial 2 Dissolved Iron (g)	Molarity of HCl (M)
1	1.002	1.131	0.1
2	1.098	1.218	0.01
3	1.18	0.961	0.001
4	1.42	1.376	0.0001
5	1.393	1.27	0.00001
6	1.407	1.369	0.000001
7	1.338	1.389	0

According to the data that was collected during this experiment, the iron bioavailability was the lowest for the pH levels ranging from 1 to 3. Iron bioavailability is the amount or percent of iron that was absorbed. This was unexpected because these are the pH levels that are normal for stomach acidity. The pH level of 1 of both Trial 1 and Trial 2 had an average absorption of 1.0665 grams out of 10 grams of iron. This resulted in an average bioavailability of 10.6%. The pH level of 2 had higher absorption, but only by .0195 grams. PH 2 had an average absorption of 1.158 grams out of 10 grams of iron. This is an average of 11.54% bioavailability. The pH of 3 had very unexpected results. In the first trial, it absorbed 1.18 grams, but in trial 2 it only absorbed .961 grams. This was also tested again and the same results occurred. PH 3 had an average absorption of 1.0705 grams with an average bioavailability of 10.7%, which was expected to be a substantially higher number.

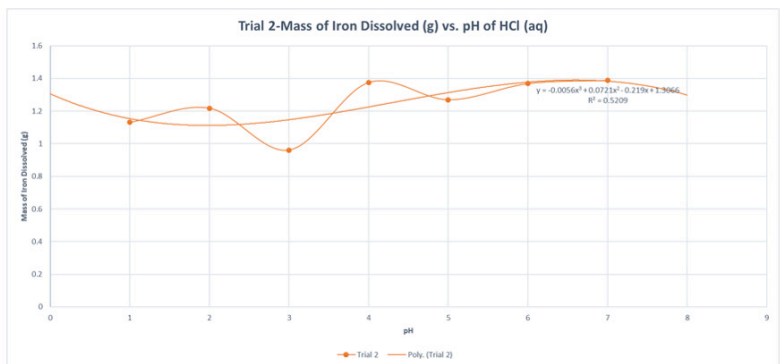
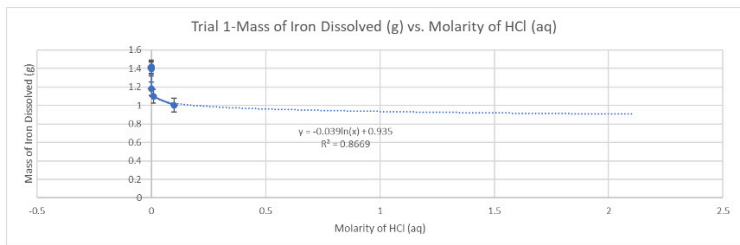
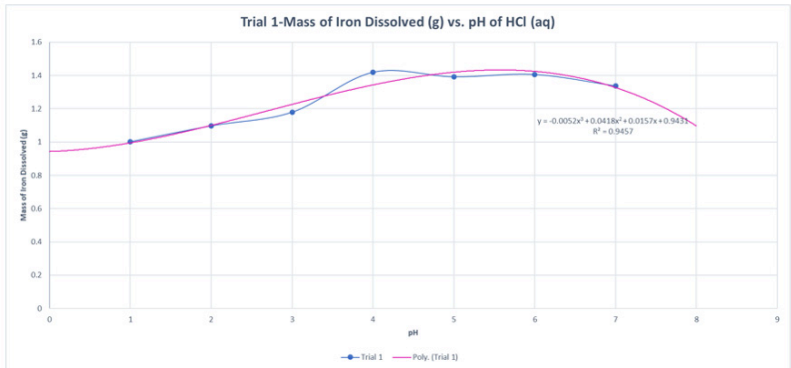
The data that was collected shows that the iron bioavailability was higher for the pH levels of 4 to 7. This was unexpected because of

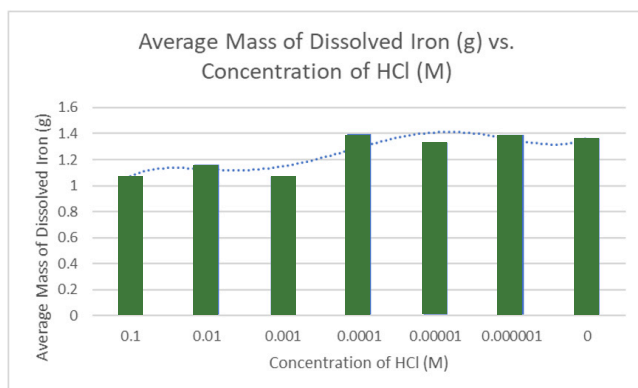
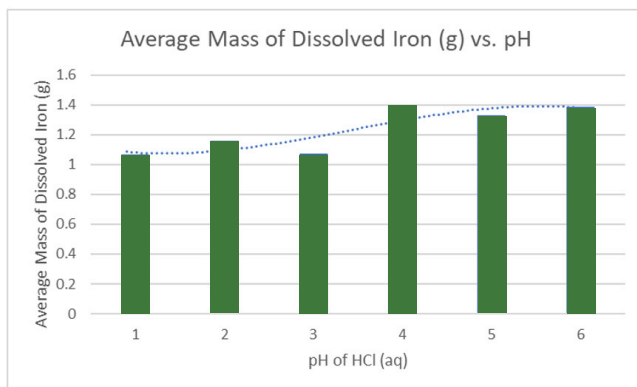
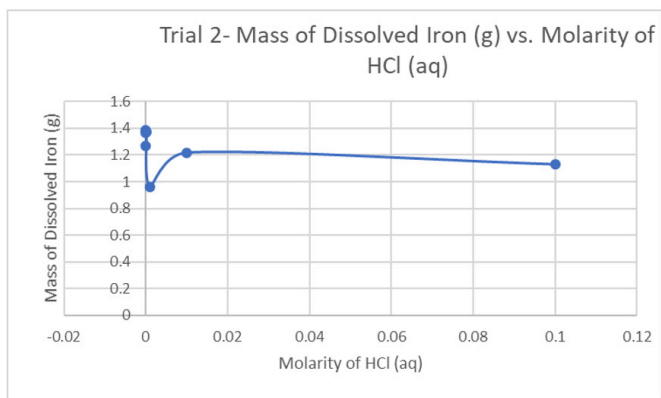
the decrease of acidity for the pHs. This pH level would be very harmful to the body if it was ever experienced and could cause digestive difficulties and severe health problems. However, PH 4 had an average absorption of 1.398 grams of iron, which was much higher than expected. It was expected that the iron absorption would decrease at this pH level because of the decline in acidity. The bioavailability of the pH of 4 averaged 13.8%. From this pH level and on they continued to maintain a high level of bioavailability. The pH 5 trials averaged 1.3315 grams of iron with an average bioavailability of 13.31%. PH 6 had the highest bioavailability level of 14.38%. It absorbed 1.588 grams of iron. The pH of 7 had decreased compared to the pH of 6, but it still didn't reach the low levels of pH levels of 1 to 3. The pH of 7 had an average absorption of 1.3625 grams of iron with a bioavailability average of 13.61%.

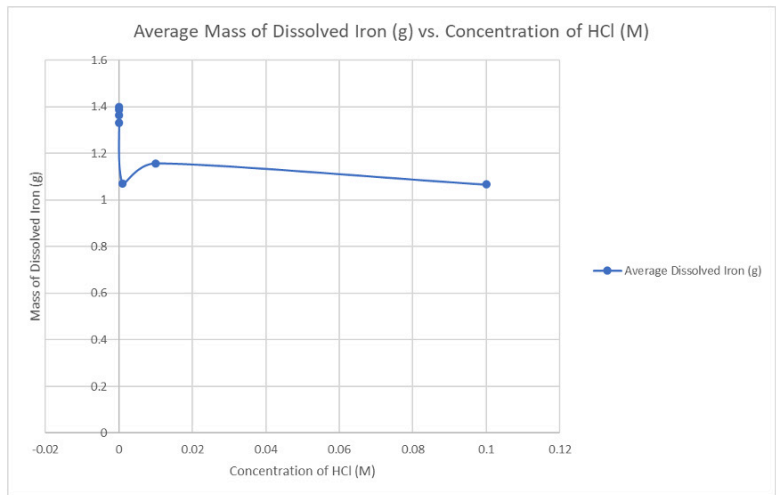
Overall, trial 1 showed better and more consistent results than trial 2. Trial 2 had a trend line of $y = -0.0056x^3 + 0.0721x^2 - 0.219x + 1.3066$, with $r^2 = 0.5209$ while trial 1 had a trend line of $y = -0.00052x^3 + 0.0418x^2 + 0.0157x + 0.9431$, with $r^2 = 0.9457$. The trend line was used to show the predictions and trial 1 had almost a perfect prediction of what was expected to happen. Overall, in this experiment, there was an impact on the iron bioavailability according to the pH level. The iron bioavailability was expected to be under 5% for the top levels but ended up being near the average absorption percent. This means that taking gastroesophageal medication is not the cause of Iron Deficiency Anemia, because these medications decrease the acidity of the stomach causing the pH of the stomach to increase, which therefore increases the bioavailability of iron.

This proves that my hypothesis was completely incorrect. I hypothesized the opposite of the actual results of this experiment. To put this into perspective, in a bowl of cereal a person will consume 4.5 milligrams of iron and only actually absorb 0.495 milligrams of that iron consumed. This means that a person's average iron intake per day is a

little under 1.5 milligrams when the pH of their stomach is under 3.5. If a person ate a bowl of cereal with the same amount of iron, but the pH of their stomach was above 3.5, then they would actually absorb 0.585 milligrams of iron.







Discussion

In this experiment, I tested the iron bioavailability in different pH levels to see where iron is most absorbed and whether the pH level was actually changing the bioavailability. In past research, any pH level above 3.5 stops the absorption of iron. The stomach rarely goes above this pH level, but for those with gastroesophageal reflux, it is more common. It is very common for someone who takes gastroesophageal reflux medication to be found with iron deficiency anemia. They may say that these two things are found together in most cases, but after performing this experiment, my opinion is that the medication does not cause iron deficiency after this experiment.

In this experiment, I hypothesized that as the pH of the stomach becomes less acidic, the gastroesophageal reflux medication will decrease the acidity of the stomach even more which will decrease the bioavailability of iron. My hypothesis was proven incorrect because my data shows that as the pH level became less acidic, which is what the gastroesophageal reflux medication would help accomplish,

the bioavailability of iron increased. I had thought that the iron bioavailability would be the greatest at the pH levels ranging from 1 to 3; however, this was the opposite of what actually happened. The pH levels ranging from 4 to 7 had a tremendous increase in bioavailability than the lower pH levels. Iron in the pH levels of 4 to 7 absorbed an average of 3% more. This means that the gastroesophageal reflux medication is not the culprit for iron deficiency anemia found in people who take gastroesophageal reflux medication. These medications actually cause the iron in the stomach to absorb more, which would do the opposite of creating an iron deficiency problem. I believe that there are enzymes in the stomach that are causing a link between treated acid reflux and iron deficient anemia.

This experiment proved to be very difficult to fulfill. There were many trials and failures in the beginning. In the beginning, we began with 100% gastric juice, which was thought to be the problem, however, the problem with the beginning trials was the filter paper that was used. In the beginning, I used qualitative filter paper which was hindering the representation of iron absorption. The other challenge that was found was making sure that the liquid was correctly heated. In the beginning trials, the hotplate was heating the liquid to almost 50°C which is 13°C more than was needed. The excess heat was causing the iron to absorb more than it would at normal body temperature. With this problem, I put the liquid in a water bath that maintained the temperature needed.

Conclusions

In conclusion, my hypothesis was incorrect. I hypothesized that as the pH of the stomach becomes less acidic, the gastroesophageal reflux medication will decrease the acidity of the stomach even more which will decrease the bioavailability of iron. The bioavailability of the iron actually increased as the pH increased. The relationship between pH and the bioavailability of iron did not seem to be a linear relationship.

A similar study completed with soil, exhibited results very similar to the experimental results of this study. In the soil study by M.C. Chuan, G.Y. Shu, and J.C. Liu, they tested the redox potential and the pH which both greatly affected the metal solubility in the soil. Metal solubilities were higher under slightly acidic conditions referring to the pH of 5 or around that area. Possible issues that may have affected my results include the inconsistency of the water temperature, inconsistent stirring and the size of the iron particles which were tested.

If I were to take this project again, I would test Fe^{+2} versus Fe^{+3} . I could potentially test the difference between lab grade Fe and food grade Fe. I could also possibly test how the different temperatures affect the bioavailability of iron. Within this project, there is an endless amount of data that could be found and that needs to be found. This topic has become something that most people are affected by, but there is not enough research done on it. ■

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