SASTA OLIPHANT SCIENCE AWARDS

SOUTH AUSTRALIAN SCIENCE TEACHERS ASSOCIATION

Prize Winner

Scientific Inquiry

Year 9-10

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Our Lady of the Sacred Heart College

INCREASE IN ACIDITY BY DIFFERENT BETAINE HCl SUPPLEMENT BRANDS.

Oliphant Science Award – Scientific Inquiry

Determining the effectiveness of different Betaine HCl supplement brands in the increasing the acidity & aiding digestion process in the stomach.

Raajvi Shah

Questioning and Predicting -

ABSTRACT

The purpose of this investigation was to determine which Betaine HCl supplement was most effective in re-acidifying the stomach. For this experiment, three different HCl supplement brands were tested (Nutricost, Vitacost, and Doctor's Best) for their efficiency in re-acidifying a diluted solution hydrochloric acid (pH 7.2 – Achlorhydria was accidentally tested) instead of (pH 4 - Hypochlorhydria). A pH probe was used to measure the solution's pH level before and after the experiment was conducted to determine if the diluted hydrochloric acid re-acidified. The most effective brand is unclear as the practical was an unfair test due to temperature and time restraints. However, the most consistent results occurred for the Nutricost supplement, as its two trials had the same temperature and starting pH. From the results obtained, it has been decided that the hypothesis is neither supported nor unsupported, as the practical was unfair, no final verdict will be made as that would be biased and would provide false information. If this was further improved to be fair, the results of this experiment would have benefitted pharmacists, doctors, and anyone needing extra digestive support as these results provide them with an understanding to make an informed and rational decision when prescribing or purchasing HCl supplements and this decision will cause an overall benefit to the patient, by enabling the patient to receive access to the best-proven supplements that will acidify stomach closest to needed pH for best digestion.

INTRODUCTION

Betaine HCl supplements are a dietary supplement used to increase gastric acid secretion, which in turn will aid the digestion of proteins. They are usually prescribed or recommended by a certified dietary physician to patients who have low levels of gastric acid, classified as Hypochlorhydria. Hypochlorhydria is a dietary condition that inhibits the absorption of nutrients and proteins in a person's body. Hypochlorhydria can be induced primarily by the long-term use of acid-neutralizing drugs such as proton pump inhibitors and antacids, by having unhealthy lifestyle habits such as drinking, smoking and by having nutrient and vitamin-deficient diet, or by contracting bacterial pathogens, e.g., *H.pylori*. Common symptoms of Hypochlorhydria are often confused with GERD, as

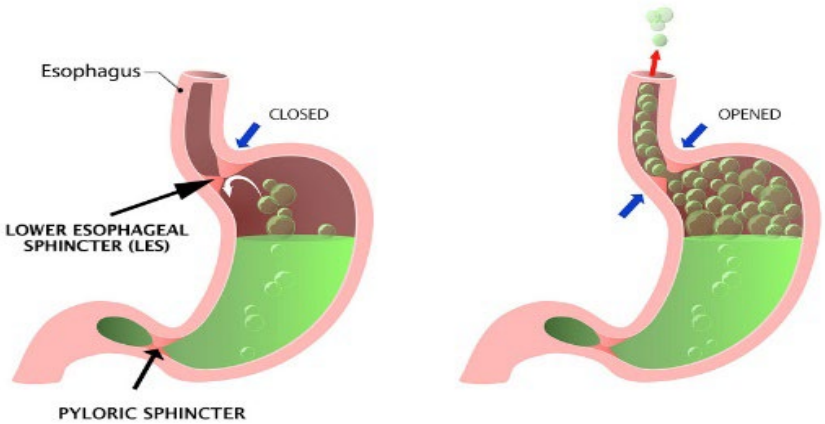


Figure 1 – Hypochlorhydria: Symptoms, causes and management tips - https://nutritionbyerin.com/hypochlorhydria-symptoms-causes-tips/

low amounts of HCl acid secretion will cause the lower oesophageal sphincter to release as the gastric acid isn't that acidic to keep it closed. Which will cause backflow of gastric acid in the oesophagus, also known as heartburn. Betaine HCl supplement tablets are found in capsule form that must be swallowed as a capsule, as the Betaine HCl activates in an aqueous environment and opening the capsule and emptying out the contents in a cup of water for easier consumption, will burn your oesophagus.

The stomach produces HCl acid in a process called gastric acid secretion. The acid is made in the gastric glands, which carry three different cells in order to make and aid the HCl acid's purposes – to break down nutrients and proteins while killing off pathogens and microbes that may have entered orally, to prevent stomach infections.

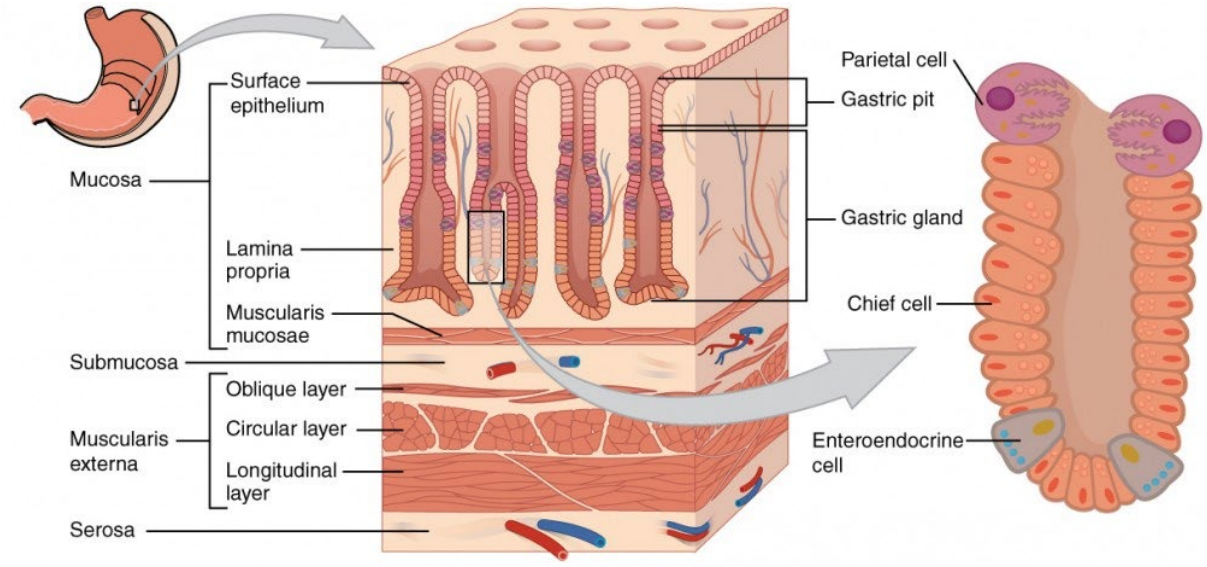


Figure 2 – The Stomach, Anatomy & Physiology - https://courses.lumenlearning.com/suny-ap2/chapter/the-stomach/

The three cells are – mucous cells, parietal cells, and chief cells. Mucous cells are typically found near the opening of the gastric gland, known as the gastric pit, and they secrete mucous to protect the stomach's lining from hydrochloric acid. The parietal cells secrete hydrochloric acid into the stomach lumen to prepare the stomach for digestion. And the chief cells secrete an inactive enzyme known as pepsinogen into the stomach lumen – this enzyme turns into pepsin when it comes in contact with the hydrochloric acid and aids the hydrochloric acid in protein breakdown.

There are three different ways in which gastric acid secretion is simulated. One of which is where gastric acid secretion is initiated by the vagus nerve, which sends down a hormone called Acetylcholine that initiates the gastric acid secretion process[1]. However, in some cases, acid secretion occurs at a lesser rate than normal, in that case, bacterial overgrowth can start to occur in the stomach, and the GI tract as undigested foods will begin to ferment, leaving the stomach prone to other bacterial pathogens such as H.pylori[11].

The ingredients found in Betaine HCl supplements aid with increasing the acidity of the stomach to allow for an increase in digestion, which in turn will prevent bacterial overgrowths. When a patient swallows and consumes the Betaine HCl supplement, the ingredients will start to reacidify the stomach temporarily, causing the pH of the stomach to increase, allowing for more effective digestion

to occur. Doctor's Best, Vitacost and Nutricost are three examples of Betaine HCl supplements as they carry the direct source of HCl, which reacts with the aqueous environment found in the lumen and steadily releases hydrochloric acid.

Gastric acid secretion prepares the stomach for the food that is about to enter the stomach through the oesophagus in bolus form. But once the food reaches the bolus, the stomach uses both chemical digestion (HCl acid secreted by gastric glands) and mechanical digestion to turn that bolus into chyme (broken food combined with some amounts of HCl acid).

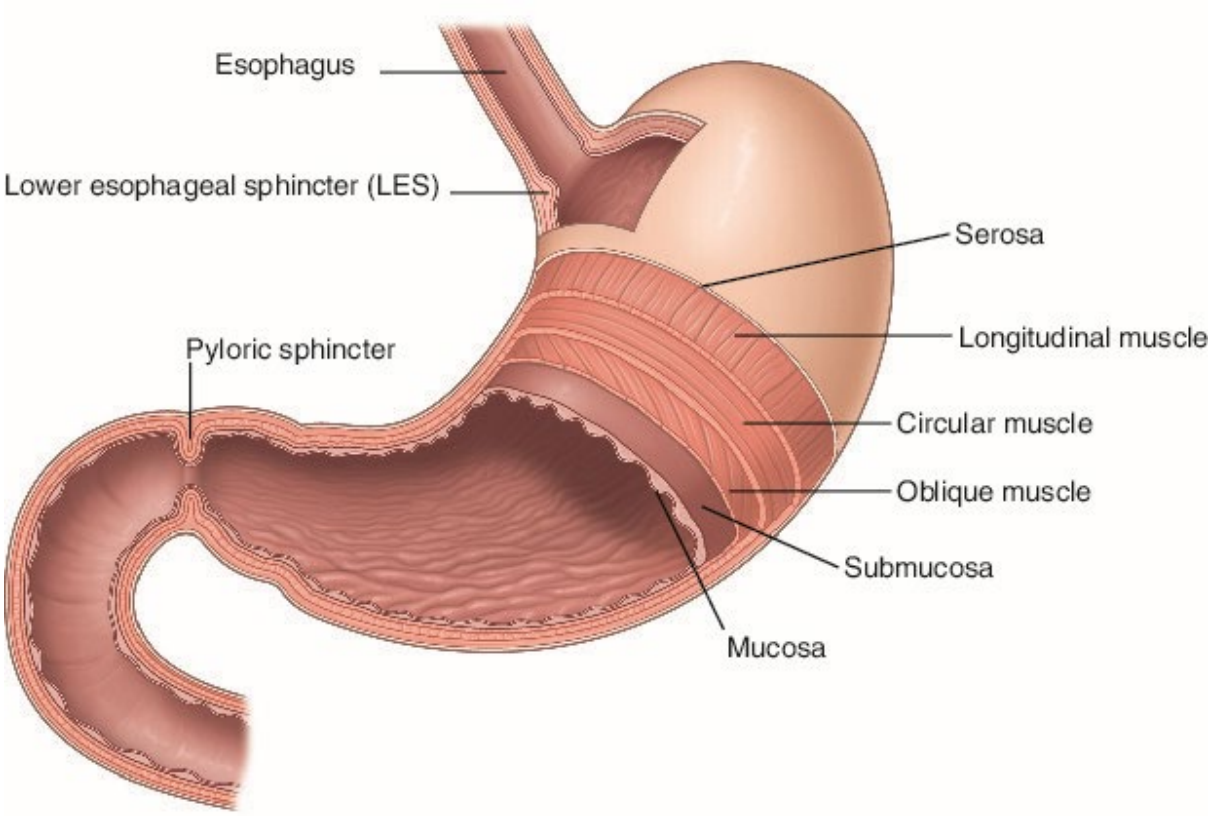


Figure 3 – The muscularis externa of the Stomach https://www.tabers.com/tabersonline/view/Tabers-Dictionary/765922/all/stomach

Mechanical digestion is the physical movement that the stomach muscles make to churn the bolus in the stomach lumen. The Muscularis Externa consists of the inner oblique layer, the middle circular layer and the longitudinal layer, these muscle layers are directed by the nerve connections found between the middle layer and the longitudinal layer, which allow them to move to breakdown foods and direct chyme towards the pyloric sphincter so the chyme can be extracted of nutrients as it enters the GI tract through the duodenum.

Planning & Conducting -

AIM

To determine which Betaine HCl supplement carries the most effective concentration of ingredients to increase acidity.

HYPOTHESIS

It is hypothesized that when each brand of Betaine HCl supplement are tested, the supplement that decreases the pH most will be the most effective, hence why Vitacost's supplements will be the most effective because the aiding ingredient, Pepsin, has a higher concentration in each capsule of that supplement, hence it will be more effective in increasing the acidity.

VARIABLES

Independent

The brand of Betaine HCl supplement being used – Doctor's Best, Nutricost and Vitacost.

Dependent

The pH of the diluted acid solution after reaction.

Controlled

1. Amount of HCl (1mL) which was diluted further to the concentration of 0.001 mL.
2. Molarity of HCl (1M)
3. Size of conical flask (250mL)
4. Same pH probe
5. Method of Stirring to dissolve (with hand holding neck of flask)
6. Temperature (should have been 37°C)
7. Same concentration of Betaine HCl in all brands.

Control

1. The control used for this experiment was the pH and molarity of the initial, undiluted HCl acid, – (pH 0, 1M).

METHOD

1. 1.25 L of tap water was to set to boil in a kettle.
2. A hot plate was pre-heated to 50°C.
3. All three supplement brands were set up with one mortar, one circular filter paper and three conical flasks in front of each brand.
4. A capsule of each supplement brand was cut open in the mortar corresponding to their container.
5. 10 mL of HCl acid (pH 0, 1M) was measured out in a beaker.
6. 99 mL of distilled water was measured out into a measuring cylinder and perfected in measurement using a clean pipette.
7. To create the first diluted solution, 1mL of HCl acid was measured out from the beaker using a clean pipette and was dropped into the measuring cylinder containing 99 mL of distilled water.

8. After the kettle was done heating the water, the temperature was measured using a thermometer. When the temperature was 99°C, the kettle was left for 10 minutes to lower the temperature.
9. During the 10 minutes in which the kettle was resting, 10 mL of the first diluted solution was measured out using a clean pipette and 10 mL measuring cylinder; and was dropped in a conical flask.
10. Step 9 was repeated eight more times for the remaining eight empty conical flasks.
11. After 10 minutes of rest, the heated water's temperature was measured again using a thermometer. It was 89°C, so, the heated water was poured out in small amounts, and normal temperature tap water was added back into the kettle to the 1.25L increment, this decreased the temperature faster.
12. The second diluted solution was created, once the heated water's temperature reached 38 degrees Celsius. 90 mL of this heated water was measured out into a clean measuring cylinder and was poured into the first conical flask for the first supplement brand (Doctor's Best).
13. The first conical flask with the second diluted solution was set atop the pre-heated hot plate, and the new temperature and pH were recorded using another clean thermometer and a pH probe.
14. The circular filter paper was shaped into a funnel and placed at the neck of the conical flask, the mortar, which included the Doctor's Best's supplement powder, was emptied into the solution.
15. Once fully emptied, the filter paper was removed, and the conical flask was held slightly aloft by the neck and was gently agitated until the solute was dissolved.
16. The conical flask was set back on the hot plate, and the new temperature and pH was recorded using a clean thermometer and pH probe.
17. Steps 4 & 12-16 were repeated again for the same supplement brand.
18. Steps 4 & 12-17 were repeated for the remaining two supplement brands.

This method was inspired by Sienna Hills' 2021 Oliphant Science Award Entry. She had recommended me to read through her research as I had trouble finding a method for Betaine HCL experimentation. After reading her research and her resource for choosing her method, I created my method. "Acid Neutralization by Different Brands of Antacids". (Sienna Hill, 2021) https://www.oliphantscienceawards.com.au/files/4320_0462-008_hill_written_report.pdf

Materials and Equipment-

MATERIALS LIST

- 9 x 250ml Conical Flasks
- 2 x 100ml Measuring Cylinders
- 1 x 10ml Measuring Cylinder
- 3 x Mortars
- A pH Probe.
- A hot plate.
- 3 x Circular shaped Filter Papers
- 3 different HCl supplement brands (Nutricost, Vitacost and Doctor's Best)

- A Pen and a logbook
- 1 x 1.25L tap water.
- 2 x 100mL bottles of distilled water
- 10 ml x HCl acid (pH 0, 1M)
- 5 x Pipettes
- 1 x 100ml Beaker
- 2 x Thermometers
- 1 x Lab Coat
- 1 x Safety Goggles.

FAIR TEST?

This experiment was not a fair test because the method for reducing the temperature of the heated water was inconsistent and not repeated. Also, the amount of heated water being disposed of each time was not recorded due to time constraints.

RISK ASSESSMENT

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☐ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: _____ ID:_____

SCHOOL: _____

Activity: Give a brief outline of what you are planning to do.

This investigations' purpose is to determine which brand of Betaine HCl supplement will best acidify the stomach acid. The lowly acidic stomach acid will be recreated through the use and dilution of hydrochloric acid (pH 0, 1M), each trial will be gently agitated using the same swirling motion made by the hand for the solute to dissolve into the solution. A pH probe will be used to measure the initial pH of the diluted solution and the pH of the solution after the reaction occurs. The brands being tested are Doctor's Best, Nutricost and Vitacost.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Type of Risk?	What is the risk?	How can I control the risk?
Chemical Risk – Used and Produced Hydrochloric acid (aq) <3 (<10% wt/wt.)	Higher Concentrations (<3 (<10% wt/wt.) if not handled with care, it can cause serious hazards and fatal risks, such as irreversible damage or fatal risks to internal organs such as lungs upon inhalation of vapour, skin, and eyes upon direct contact, as it is a corrosive acid.	Avoid inhalation of vapour and follow standard handling procedures. Do not touch directly, be careful when handling, and avoid experimenting with HCl and store only small amounts. Keep away from other chemicals (especially incompatible) materials such as oxidizing agents, organic materials, metals, and alkalis. HCl can corrode through metal surfaces.

Physical Risk - Water <43.5°C (cold-warm) & >50°C (lukewarm – hot)	Harmful when in direct contact and consumption when >50°C. Harmful when being tested in a laboratory. Generally, water below 43.5°C is considered safe for adults and children, but prolonged exposure to cold water can cause numbness and in worse cases, hypothermia.	Do not drink the water in a laboratory as it could be contaminated with harmful, chemical mist and vapours. Keep safe by not touching the water when it is above 50°C as it will cause major burns. Water spilled on the floor is a slipping hazard.
Chemical Risk - HCl supplements (Doctor's Best, Nutricost, Vitacost)	Consumption without recommendation of a certified physician for assumed or not tested diagnosis can cause problems such a burning sensation in the chest area (heartburn). Larger amount consumption can burn the stomach lining.	Do not consume unless you have been diagnosed with Hypochlorhydria, Achlorhydria or have been recommended by a certified physician. Especially do not consume is a laboratory as it now prone to chemical contamination.
Physical Risk – GLASSWARES - Alcohol Thermometer, Beakers (2 x 100ml), Conical Flasks (9 x 250ml), Measuring Cylinders (2 x 100ml) & (1 x 10ml)	If not handled properly, the thermometer will break and risk the experiment conductor and others in the vicinity of being injured by glass fragments	Carefully sweep broken glass and glass fragments with a brush and a dustpan. Do not attempt to use fingers.
Pipettes (5 x 4ml)	If not handled properly, the chemical that is filled in the pipette could eject out and cause the chemical to come in direct contact with yourself and other people surrounding the experiment. Can be confused with other pipettes used, which can lead to contamination of chemicals and unknown reactions.	Make sure to separate used and unused pipettes and handle the pipette with care.
pH electrode (pH probe – glass electrode and pH sensor)	The glass electrode of the pH probe could break which can release the toxic chemicals used to help its function.	If the glass electrode snaps, clean up the fragments using a brush and a dustpan. Do not attempt to pick up glass fragments with your fingers.
Mortar (came with pestle)	When broken can cause deep wounds and cut	Carefully discard broken pieces.
Electric Hotplate		

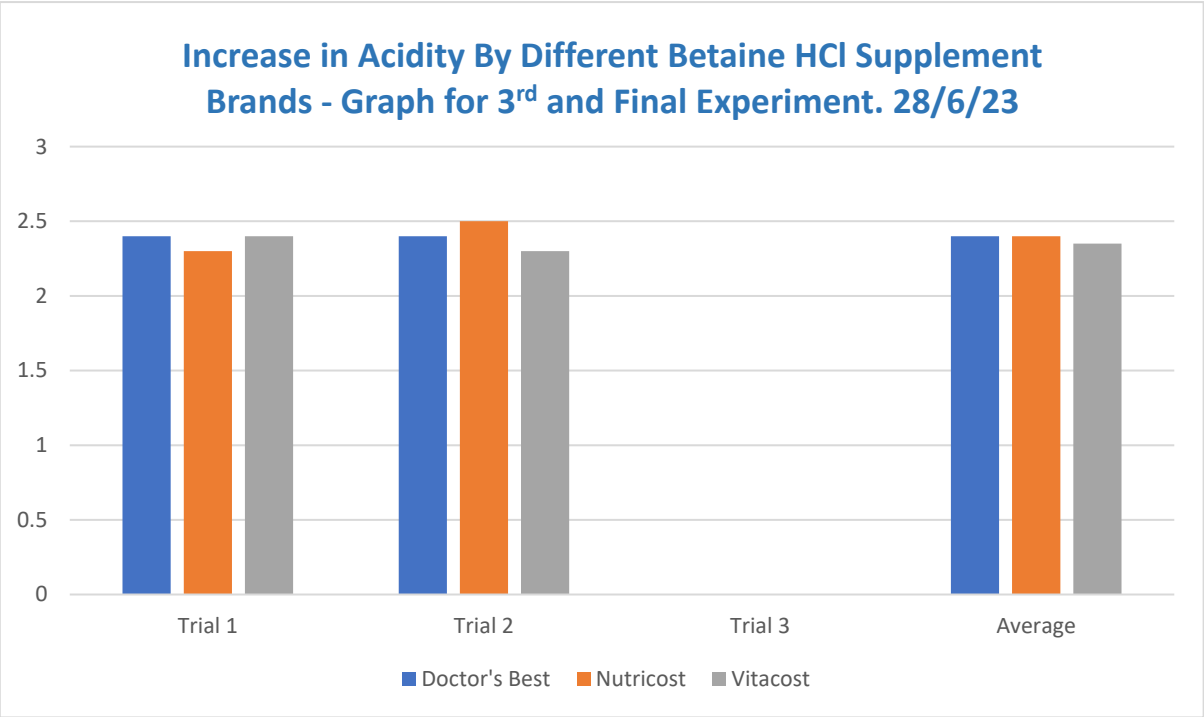
	This is an ignition source. Therefore, do not let flammable liquids sit atop its surface when turned on and off, when its isn't certified as spark proof. Burns can occur when hot plate is in direct contact with skin whilst the hotplate is turned on and afterwards as the hot plate retains heat. The electrical cord could also get damaged by heat and cause an electric shock	Inspect cord regularly for signs of damage this could be that the cord is loose in the plug, the cord is loose at the entry to hot plate, or that the cord may have signs of corrosion or other damage. The hot plate must be tested and tagged at regular intervals by an adult or a lab technician. Ensure that the hot plate's cord is made of heatproof material. Do not touch hot plate when turned on and after it is turned off.
Electric Kettle	This is ignition source on the inside, no other liquid must be placed in the kettle other than water, as chemical mists and vapour will be released and inhalation of those is harmful to health. The body of the kettle is still hot after use as the body retains heat from the liquid heated inside earlier. The electrical cord of the kettle could also be damaged by heat caused through kettle and cause an electric shock. Hot water inside the kettle can cause major burns when came in direct contact.	Inspect cord regularly for signs of damage this could be that the cord is loose in the plug, the cord is loose at the entry to kettle, or that the cord may have signs of corrosion or other damage. The kettle must be tested and tagged at regular intervals by an adult or a lab technician. Ensure that the kettle's cord is made of heatproof material. Do not touch kettle by the body, only use the handle when the hot water is still in there and after the water has been disposed of. Do not touch the hot water inside the kettle.
Chemical Risk – Plastic Bulb Pipette (not included in final materials list as it was not used in the last experiment but was used briefly in the 2nd experiment.)	Ingestion can occur if one uses their mouth to fill the pipette from the top. Organic solvents may swell the surface layer of plastic, which could cause cracking and leaking of the pipette.	Do not use mouth to fill pipette. Always use a properly fitted bulb to fill the pipette. Do not fill pipette with organic solvents and do not rinse and clean pipette with organic solvents.

Process, Analysis & Communication -

RESULTS

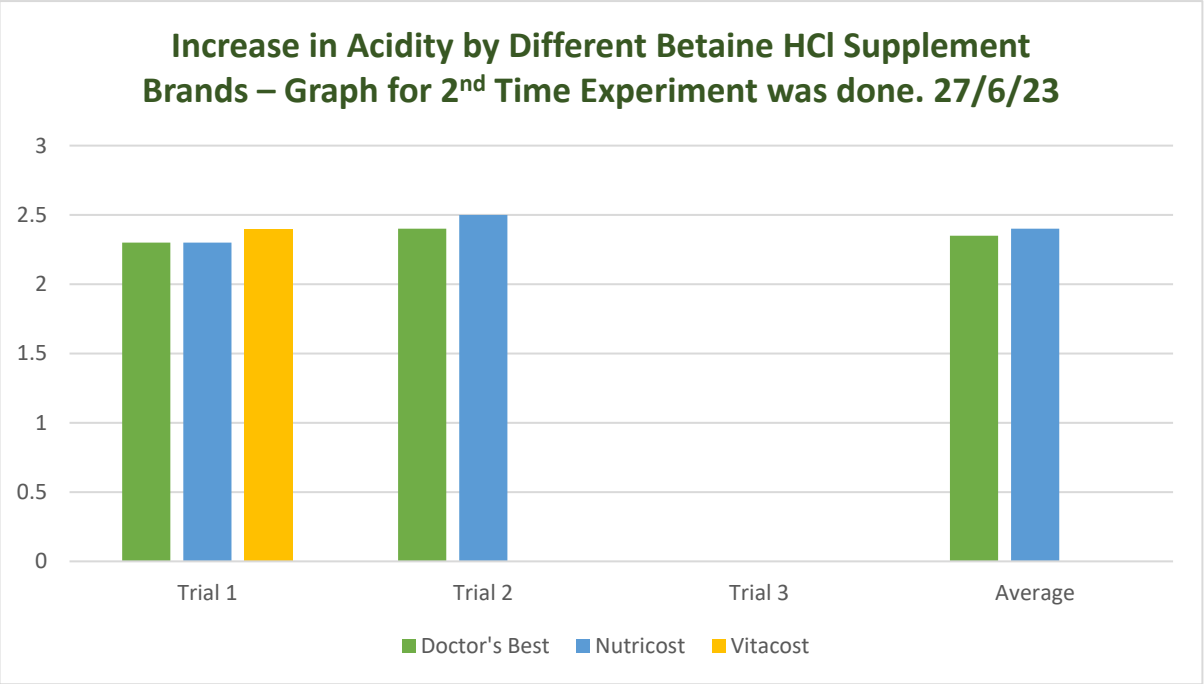
Increase in Acidity by Different Betaine HCl Supplement Brands – Table for 3rd and Final Experiment. 28/6/23

	pH and Temperature of diluted HCl solution after reaction occurred.		
Brands of Supplements	**Trial 1**	**Trial 2**	**Trial 3**
Doctor's Best	Initial pH - 7.2 Initial Temp – 37°C. After reaction pH – 2.4 After reaction temp – 38 degrees Celsius.	Initial pH - 7.2 Initial Temp – 32°C. After reaction pH – 2.4 After reaction temp – 34°C.	
Nutricost	Initial pH - 7.2 Initial Temp – 32°C. After reaction pH – 2.3 After reaction temp – 33°C.	Initial pH - 7.2 Initial Temp – 32°C. After reaction pH – 2.5 After reaction temp – 34°C.	
Vitacost	Initial pH - 7.2 Initial Temp – 32°C. After reaction pH – 2.4 After reaction temp – 33°C.	Initial pH - 7.2 Initial Temp – 39 degrees Celsius. After reaction pH – 2.3 After reaction temp – 40°C.	



Increase in Acidity by Different Betaine HCl Supplement Brands – Table for 2nd Time Experiment was done. 27/6/23

	pH and Temperature of diluted HCl solution after reaction occurred.		
Brands of Supplements	**Trial 1**	**Trial 2**	**Trial 3**
Doctor's Best	Initial pH - 6.7 Initial Temp – 32°C. After reaction pH – 2.3 After reaction temp – 34°C.	Initial pH - 7.2 Initial Temp – 32°C. After reaction pH – 2.4 After reaction temp – 34°C.	
Nutricost	Initial pH - 7.2 Initial Temp – 32°C. After reaction pH – 2.3 After reaction temp – 33°C.	Initial pH - 7.2 Initial Temp – 32°C. After reaction pH – 2.5 After reaction temp – 34°C.	
Vitacost	Initial pH - 7.2 Initial Temp – 32°C. After reaction pH – 2.4 After reaction temp – 33°C.		



Chemical Reaction Between Betaine HCl and pH 7.2 (neutral) solution-

Supplements Brands & Concentration of Active Ingredient	Reactants -> Product	Balanced Equation
Doctor's Best (650mg) Nutricost (650mg) Vitacost (650mg)	Betaine HCl (s) + Water (aq) -> **Betaine (aq) + HCl (aq)**	

(s) – Solid, (aq) – Aqueous

The initial pH of the solution, the final pH after reaction & when the practical ended (3rd and Final testing) =

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Evaluating -

DISCUSSION

The practical conducted was to determine the most effective brand in acidifying low-level stomach acid, this meant a comparison occurred among three different supplement brands, Doctor's Best, Nutricost and Vitacost. The most effective brand of Betaine HCl supplement is unclear, as this practical is an unfair test due to temperature changes, and only 2 trials were done per supplement. But Vitacost's (650mg Betaine HCl) & (162mg Pepsin) supplements' results have been able to increase acidity slightly better to the average of pH 2.35, whereas the Doctor's Best supplements and Nutricost supplements have both been able to increase acidity to the average pH of 2.4.

This practical was an unfair test due to different temperatures occurring, as it was unclear whether the last trial done for Vitacost was slightly decreased by the temperature or by the Betaine HCl, as Doctor's Best's trials were similar to that of Vitacost's - Doctor's Best 1st trial (28/6/23) had the initial temperature of 37°C and the final pH of 2.4, and the 2nd trial (27/6/23) had the initial temperature of 32°C and the final pH of 2.4 as well. Therefore, coming to a final verdict as to which supplement was most effective in increasing acidity would be biased and would be false information.

The purpose of the control (HCl) for this experiment was to recreate the stomach acid and to prove that Betaine HCl supplements are crucial in the acidification of low stomach acid, the control was diluted fairly, as the pH was consistently 7.2, which means that a systematic error occurred, as the calculations made in the method were to achieve the pH level of 4, which recreates the gastric disorder, Hypochlorhydria (low-level secretion of gastric acid). But that the practical inadvertently tested Achlorhydria (inability to secrete any HCl acid).

Another systematic error made in the practical was the temperatures in both 2nd and 3rd runs of the experiment, in the 2nd run (27/6/23), since there wasn't an exact method to get a temperature of 37°C, an improvisation was made, and 1.25L of water was heated in a kettle was poured out in small quantities and normal temperature water was added back to the 1.25L increment line to decrease the temperature. This was a satisfactory method to decrease the temperature, but it wasn't precise. Therefore, this method was not repeatable the next day (28/6/23 3rd experiment run), when the temperature was to be recreated to match the day before so that the experiment would at least be a fair test, even if the temperature of 37°C was not achieved, it couldn't be recreated, therefore making the entire practical an unfair test.

If this practical was to be repeated, definite improvements would have to be made to ensure that is practical is a fair test and is repeatable. An improvement to decrease the temperatures consistently to 37°C would be to implement an exact amount of heat water millilitres to dispose out and add back in the same number of millilitres that was disposed of but as normal temperature water, as this ensures that the same method can be repeated and the temperature of 37°C can be reached every time the practical is conducted to aid with the accuracy and reliability of the practical. E.g., the temperature of the hot water was recorded - 100°C. 25 mL of hot water was disposed and substituted with 25 ml of normal temperature water, 4 times, and that decreased the temperature to 37°C.

In addition, further trials of each supplement should be conducted if this practical is repeated, as more trials will increase the accuracy and reliability of the practical and will decrease the effect of the systematic errors that were made.

Ultimately, the calculation of the pH levels for 4 could have been also further researched, as more research and experimentation of pH levels could lead to achieving the perfect series of serial dilutions to reach the pH of 4 for the initial solution.

And a last improvement that could be made to the method to determine which supplement is most effective is to time how long each supplement's acidification effect takes to wear off after acidification has occurred, as this will provide more in-depth information about each supplement and based on the results of the two factors – average pH given by each supplement and the average time the solution took until the effects of the Betaine HCl supplement wore off, will give provide the experimenter with more information to analyse and decide which supplement was most effective.

CONCLUSION

The aim of this investigation was to determine which brand of Betaine HCl supplement best acidifies a stomach with low levels of gastric (HCl acid). In this practical, three different brands of Betaine HCl supplements were tested (Doctor's Best, Nutricost, and Vitacost) were tested for the effectiveness of re-acidifying diluted HCl acid (technically neutralised HCl acid as the pH was 7.2) The supplements were cut open in a mortar and added to the diluted solution. The supplement powder and the solution were gently agitated together to imitate the churning of the stomach, and the pH was measured after the powder was dissolved. Two trials were conducted for each supplement brand.

From the results obtained, the practical was an unfair test, and Hypochlorhydria was not tested (Achlorhydria was). Therefore it was concluded that a final verdict of if the hypothesis supported or didn't support the results of the most effective Betaine HCl supplement brand would be a biased and false statement. Certified physicians, pharmacists and doctors can hopefully gain an understanding as to how Betaine HCl supplementation can aid digestion among patients who show signs of Hypochlorhydria and Achlorhydria, and that Betaine HCl supplements can effectively increase acidity levels.

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OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☐ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: Raajvi Shah ID:_____

SCHOOL: Our Lady of the Sacred Heart College

Activity: Give a brief outline of what you are planning to do.

This investigations' purpose is to determine which brand of Betaine HCl supplement will best acidify the stomach acid. The lowly acidic stomach acid will be recreated through the use and dilution of hydrochloric acid (pH 0, 1M), each trial will be gently agitated using the same swirling motion made by the hand for the solute to dissolve into the solution. A pH probe will be used to measure the initial pH of the diluted solution and the pH of the solution after the reaction occurs. The brands being tested are Doctor's Best, Nutricost and Vitacost.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Type of Risk?	What is the risk?	How can I control the risk?
Chemical Risk – Used and Produced Hydrochloric acid (aq) <3 (<10% wt/wt.)	Higher Concentrations (<3 (<10% wt/wt.) if not handled with care, it can causes serious hazards and fatal risks, such as irreversible damage or fatal risks to internal organs such as lungs upon inhalation of vapour, skin, and eyes upon	Avoid inhalation of vapour and follow standard handling procedures as in, do not touch direct, be careful when handling, and avoid experimenting HCl with, store only small amounts. Keep away from other (especially incompatible) materials such

	direct contact, as it is a corrosive acid.	as oxidizing agents, organic materials, metals, and alkalis. HCl can corrode through metal surfaces.
Physical Risk - Water <43.5°C (cold-warm) & >50°C (lukewarm – hot)	Harmful when in direct contact and consumption when >50°C. Harmful when being tested in a laboratory. Generally, water below 43.5°C is considered safe for adults and children, but prolonged exposure to cold water can cause numbness and in worse cases, hypothermia.	Do not drink the water in a laboratory as it could be contaminated with harmful, chemical mist and vapours. Keep safe by not touch the water when it is above 50°C as it will cause major burns. Water spilled on the floor is a slipping hazard.
Chemical Risk - HCl supplements (Doctor's Best, Nutricost, Vitacost)	Consumption without recommendation of certified physician for assumed or not tested diagnosis can cause problems such a burning sensation in the chest area (heartburn). Larger amount consumption can burn the stomach lining.	Do not consume unless you have been diagnosed with Hypochlorhydria, Achlorhydria or have been recommended by a certified physician. Especially do not consume is a laboratory as it now prone to chemical contamination.
Physical Risk – GLASSWARES - Alcohol Thermometer, Beakers (2 x 100ml), Conical Flasks (9 x 250ml), Measuring Cylinders (2 x 100ml) & (1 x 10ml)	If not handling properly, the thermometer will break and risk the experiment conductor and others in the vicinity of being injured by glass fragments	Carefully sweep broken glass and glass fragments with a brush and a dustpan. Do not attempt to use fingers.
Pipettes (5 x 4ml)	If not handled properly the chemical that is filled in the pipette could eject out and	

	cause the chemical to come in direct contact with yourself and other people surrounding the experiment. Can be confused with other pipettes used which can lead to contamination of chemicals which can lead to unknown reactions.	Make sure to separate used and unused pipettes and handle the pipette with care.
pH electrode (pH probe – glass electrode and pH sensor)	The glass electrode of the pH probe could break which can release the toxic chemicals used to help its function.	If the glass electrode snaps, clean up the fragments using a brush and a dustpan. Do not attempt to pick up glass fragments with your fingers.
Mortar (came with pestle)	When broken can cause deep wounds and cut	Carefully discard of broken pieces.
Electric Hotplate	This is an ignition source, therefore do not let flammable liquids sit atop its surface when turned on and off, when its isn't certified as spark proof. Burns can occur when hot plate is in direct contact with skin whilst the hotplate is turned on and afterwards as the hot plate retains heat. The electrical cord could also get damaged by heat and cause an electric shock	Inspect cord regularly for signs of damage this could be that the cord is loose in the plug, the cord is loose at the entry to hot plate, or that the cord may have signs of corrosion or other damage. The hot plate must be tested and tagged at regular intervals by an adult or a lab technician. Ensure that the hot plate's cord is made of heatproof material. Do not touch hot plate when turned on and after it is turned off.
Electric Kettle	This is ignition source on the inside, no other liquid must be placed in the kettle other than	Inspect cord regularly for signs of damage this could be that the cord is loose in the plug,

	water, as chemical mists and vapour will be released and inhalation of those is harmful to health. The body of the kettle is still hot after use as the body retains heat from the liquid heated inside earlier. The electrical cord of the kettle could also be damaged by heat caused through kettle and cause an electric shock. Hot water inside the kettle can cause major burns when came in direct contact.	the cord is loose at the entry to kettle, or that the cord may have signs of corrosion or other damage. The kettle must be tested and tagged at regular intervals by an adult or a lab technician. Ensure that the kettle's cord is made of heatproof material. Do not touch kettle by the body, only use the handle when the hot water is still in there and after the water has been disposed of. Do not touch the hot water inside the kettle.
Chemical Risk – Plastic Bulb Pipette (not included in final materials list as it was not used in the last experiment but was used briefly in the 2nd experiment.)	Ingestion can occur if one uses their mouth to fill pipette from the top. Organic solvents may swell the surface layer of plastic, which could causing cracking and leaking of the pipette.	Do not use mouth to fill pipette , always use a properly fit pipette filler to fill pipette. Do not fill pipette with organic solvents and do not rise and clean pipette with organic solvents.

Teacher Signature – Caroline Beekman - 

Student Signature – Raajvi Shah - 

Introduction

3/5/23

Name ~ Raajvi Shah

Category ~ Scientific Inquiry

School ~ Our Lady of the Sacred Heart College Enfield.

OSA Coordinator ~ Caroline Beekman

RULES FOR A SUCCESSFUL ENTRY :

- 2000 word limit
- My original work
- Log book journal for research, ideas, planning, experiment, results.

REPORT MUST INCLUDE :

- Questioning & Predicting - What question have I chosen to investigate, and based on my research, what is my hypothesis for my experiment. (Hypothesis is pre-experiment)
- Planning & Conducting - what is the reasoning behind my method that I will choose, what are my independent, dependent & controlled variables, is it a fair test? Can someone else recreate my experiment from the method I have written. (coherency)
- Materials & Experiment - List all materials & equipment used, do the risk assessment, how to control listed risks?
- Process & Analyse Data & Information - Present measurements & observation acquired from investigation. Depending on year level, these may include tables, graphs, photos or sketches. Analyse results. What patterns & relationships are seen in the data? What conclusions can be made from the results? Does result support hypothesis?
- Evaluating - Discuss how you can further improve your investigation (method, accuracy, organisation, planning, research) How are my →

findings beneficial to others? How can my findings help? What questions could I have asked that could have been researched on or could have been researched further about?

- Communicating - Inquiry must be presented in coherent words & suitable/appropriate scientific terms. Use graphs, charts & table to present your findings. Have a reference section/ bibliography for all sources, pictures and research used in your investigation. Quote & footnote any direct quotes used in Inquiry to avoid plagiarism.

Headings, titles, figures, captions, tables, graphs, charts, references, & log book/ journal are not included in the overall word count.

Inquiry Question Ideas

4|5|23

1. Testing Keratin strength against acids with ~~decreasing~~ pH levels from 6 - 0.
   - Hair would be tested as Keratin
   - Recording & observing hair's chemical strength.
   - Hydrogen bonds & damage.
2. Which Anti-Fungal cream is best at healing Fungal Infection?
   - Over-the-counter fungal medication
   - Would have to grow a fungal infection - which can vary when grown. (risk of getting infection)
   - Microbial growth varies per test.
3. Which HCl supplement (best) increases acidity in gastric acid for better digestion?
   - Dietary supplement - can be found OTC but before consumption, needs to be double checked by a doctor.
   - (Hypochlorhydria) used by people with low levels of gastric acid.

- risks can be contained / managed.
- Is more time efficient as supplement generally takes 6 minutes to work. (or less, if surface area is increased?)

4. Rose breeding - How to breed a hardier, prettier rose from scratch. (DO NOT COPY.)
- Can use my roses at home
- Is not time efficient as it takes a long time to germinate.
- Hybridization, pollen, parent's plants traits & function.

Chosen Inquiry

6/5

BRIEF EXPERIMENT PLANNING

3. HCl supplement, pH decrease, acidity increase in gastric acid for better digestion in a stomach with low levels of HCl acid. (Hypochlorhydria)

Aim: to determine the most effective HCl supplement brand at increasing acidity in a short span of time.

Independent Variable: Brand of HCl supplement

Dependent Variable: The effectiveness of the supplement brand e.g- amount of time, difference in pH decrease from pH 4.

Controlled Variables:
- Same pieces of equipment, with equal measurements for each testing - e.g 100 ml measuring cylinder for all.
- Same concentration of active ingredient
- Same concentration of HCl acid? (Molarity of HCl?)
- Same temperature for all solutions for testings (most likely I'll recreate stomach temp when digesting foods - for more accuracy.)

Materials & Equipment - Lab safety gear, conical flasks x4 (to avoid injuries if explosive reaction occurs, as we are working with acids), 3 brands of supplements, pH probe, Hot plate (to heat up solution)
↓
HCl

Research - GASTRIC ACID SECRETION

16/5/23

- How does it work?
- What is its function?
- What's its normal pH?
- What if its low? (secreted less)

- pH level of gastric acid is between 1.5 to 3.5
- The acid is secreted to digest foods (breakdown proteins) and kill pathogens that may have entered orally and gone down the esophagus. Before potencial pathogen spread throughout the body along with digested nutrients.
- Gastric acid's corrosive acidity is caused by Hydrochloric acid.
- HCl acid is secreted by the parietal cells in the gastric glands which are found in the surface epithelium after the gastric pits.
- Gastric pit walls are lined with mucus cells, however gastric gland walls are lined with multiple different cells which secrete HCl and pepsinogen.
- HCL is secreted through the parietal cells in the gastric gland
- In the parietal cell cytoplasm - water (H_2O) is combined with carbon dioxide (CO_2) to make carbonic acid (H_2CO_3). Carbonic acid is then catalysed by carbonic anhydrase and the carbonic acid breaks apart into a hydrogen ion (H^+) and a bicarbonate ion (HCO_3^-)
  - ↳ gets pumped in stomach lumen through the ATPase ion pump in exchange for (K^+) potassium ions.
  - gets pumped into bloodstream through anion exchanger, in exchange of (Cl^-) chloride ions.
- H^+ meets Cl^- and their opposite charges bind each other to make "Hydrochloric acid."
- Then the chloride ions get pumped out of parietal cell through the chloride channel
- Pepsinogen is an enzyme made by the chief cells found in the gastric gland.
- Pepsinogen is the inactive enzyme state of pepsin - to activate pepsin, HCl acid is essential.
- Pepsin (its activate state) is an enzyme that is able to aid HCl in protein breakdown, hence its essential for pepsinogen to be activated.

2015/23

- Together HCl & pepsin get secreted into the stomach lumen.
- HCl acid creates a very acidic environment with low pH - low pH is activator for pepsin.
- Body keep it's temperature at approx. 100°F aka 37°C, this allow all bodily functions to function properly. Therefore, digestion best occurs during this temperature.
- The stomach consists of inner oblique, circular & longitudinal (in that order) muscles layers above the submucosa, known as "the muscularis externa."
- Inner oblique layer is primarily responsible for the "churning" aka mechanical digestion that occurs in the stomach in order to break down nutrients & proteins - "chemical digestion."
- Middle circular layer uses the stomach's longitudinal axis to direct & regulate the output of chyme from the stomach through the pyloric sphincter
- Circular layer muscles build up more at the pylorus especially because of their regulating factor.
- Between the last layer of the muscularis externa series and the middle circular layer, is an area of nerve connections, which allow the muscles in that region to move & function properly on the neurons' command.
- The longitudinal layer aids the circular layer in terms of the directional movement of the chyme.
- Chyme is the broken-down food + some gastric acid that exits the stomach and continues it's journey down the small intestine
- Both mechanical & chemical digestion are needed to break down food.
- When chemical digestion isn't functioning properly, that means not enough HCl is being secreted through the gastric glands.
- Mucous cells are also found in the gastric gland, they secrete mucous to protect stomach lining from HCl.

20/5/23

Figure 1 - GERD being a symptom of Hypochlorhydria.

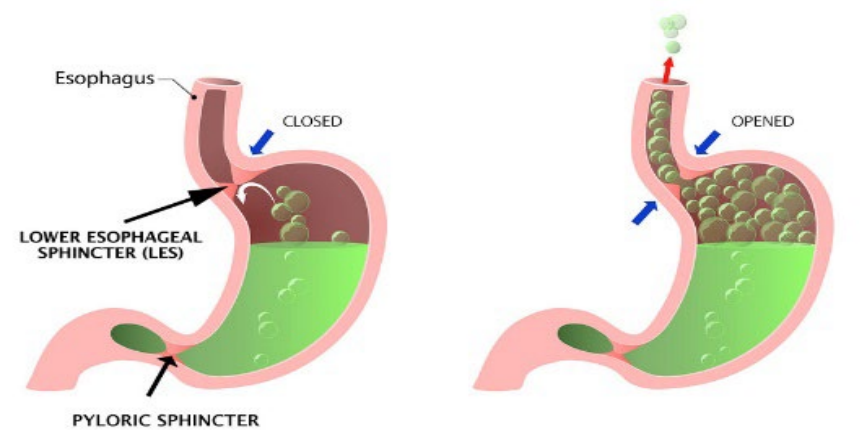


Figure 2 - Cells involved in gastric acid secretion

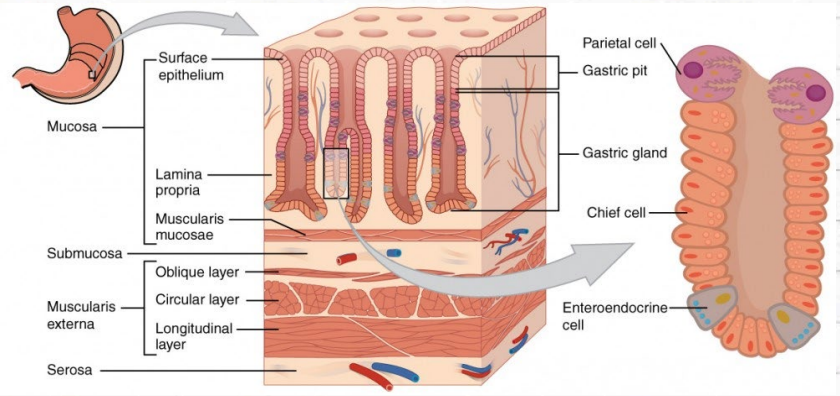
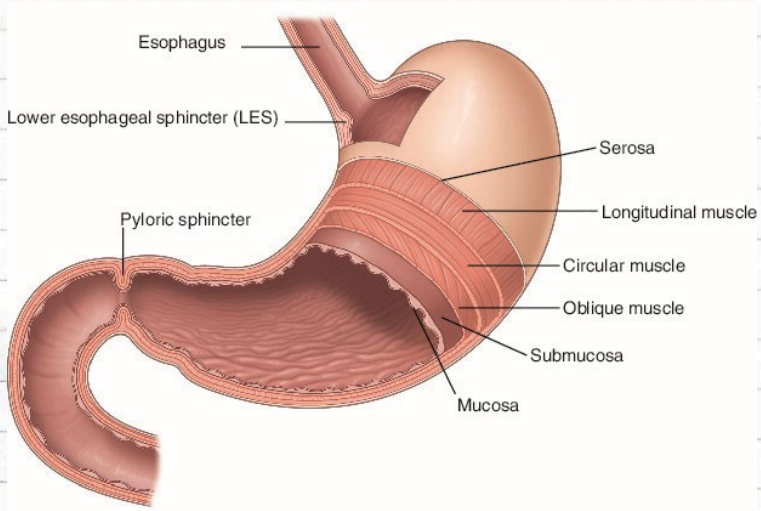


Figure 3 - The Muscularis Externa - 3 muscles.



1/6/23

Research - BETAINE HCL SUPPLEMEN

- Side effects of supplements?
- How does it work?
- Condition / disorder?
- What are they?
- What are they used for?
- Temporary or permanent treatment?
- Symptoms of the condition?

- Betaine HCl supplements are used to increase gastric acid in the stomach to improve chemical digestion. (absorption of nutrients & vitamins, minerals & proteins.)
- Betaine HCl is the primary ingredient in the supplement, but is often paired with other 'aiding' ingredients such as Pepsin & bitter tasting plant ingredients aka "bitters"
- Direct source of supplementation, by steadily releasing H^+ ions in an aqueous environment (stomach lumen). H^+ ions react with Cl^- ions and that increases the acidity in the stomach.
- Betaine HCl supplementation is recommended by doctors to patients who show signs of or have tested positively for low levels of gastric acid.
- Meaning, those patients can't secrete HCl at a normal rate:
- Betaine HCL supplements take 6 mins to work.

Things to make note of when doing experiment.

- Supplements can come in tablets or capsules and take 6 mins to work but if I'm doing these in a lunch time (probably as my teachers have told me.) & I'm testing 3 brands - and I want to trial each brand 3 times - that means the entire testings/experiment will take 54 mins as $3 \times 3 = 9$ & $9 \times 6 = 54$, but my lunch times are only 45 mins long.
- Meaning I must reduce reaction - and I can do that (this was consulted with my science teachers) by increasing my surface area - so I'd have to crush the tablet or open the capsule to increase the reaction speed.

Research

BETAINE HCl + PEPSIN SUPPLEMENT.

1/6/23

- Side effects of supplement?
- How does it work?
- Common Supplements?
- What Condition does it treat — symptoms of condition. cause of condition?
- How does this research benefit society?
- Active Ingredients & result of supplement after digestion through a chemical equation?

- Increases the acid in the stomach to improve the absorption of nutrients such as Vitamin B12, minerals and protein whilst killing pathogens & harmful microbes.
- Betaine HCL steadily increases the acidity of stomach by releasing H^+ ions in an aqueous environment.
- The pH level between 1.5 - 3 activates protease which breaks protein into amino acids, and also activates pepsin from pepsinogen, which aids protein breakdown. (That means its a safe supplementation)
- Betaine HCL + Pepsin supplements help to increase amount of (Hydrochloric acid) gastric acid.
- Hypochlorhydria is common among people over the age of 50, every decade we age our stomach acid amount lowers, making us more prone to Hypochlorhydria.
- Symptoms of Hypochlorhydria is - GERD, abdominal pain, un-digested food in feces, diarrhea, Bloating, nausea.
- GERD occurs as a symptom due to the low level of acidity in the stomach, which opens the lower esophageal sphincter and acid will regurgitate up into the esophagus, even though the acid may be less acidic, it is still harmful outside the stomach.
- Betaine alone is a vitamin-like substance found in foods like beets.
- Betaine HCL temporarily lowers pH level in stomach, and should only be taken during meal times.
- Known for being able to supplement Hydrochloric acid directly in the stomach lumen.
- Since the stomach cells in the stomach of someone with Hypochlorhydria release less H^+ ions to react with the Cl^-, the supplement upon digestion releases H^+ ions for Cl^- ions to make Hydrochloric acid.
- $H^{\oplus}$ & $Cl^{\ominus}$ are opposite charges, hence they combine to make HCl acid.

7/6/23

Method

(I want to use 100 ml solution, so I can easily understand & calculate HCl concentration with each dilution.)

(I need to play around withe the water & HCl acid ratio, so I can achieve pH of 4)

1. Measure out 95-98 ml of water in a 100ml measuring cylinder and pour into conical flask.
2. Measure out 2-5 ml of HCl acid using a pipette and drop into conical flask that is filled with water. Use pH probe to ensure pH is 4.
3. Place conical flask on a heating block, place thermometer in flask, on the heating block and set up to 40°c or 37°c to keep HCl acid at constant temp of 37°c or 40°c ?
4. Crush supplement (brand) in a mortar & pestle, and place crushed supplement & pH probe simultaneously, use a stirring rod to stir acid & supplement to imitate constant stomach churning.
5. Start stopwatch when putting crushed supplement in and record when pH reaches between 1-2. and record how long it took.
6. Repeat steps 1-5 two more times on same supplement brand to ensure the reliability of the brand.
7. Repeat steps 1-6 on the 3 other supplement brands.

Material List

- 12 X 125ml conical flasks
- 1 X measuring cylinder
- 95-98(ml) X 12 water
- 2-5 (ml) X 12 HCl acid (pH of solution = 4, (molarity??))
- Pipette
- 4 brand of supplements (Thorne, Doctor's Best, Solaray, Protocol for Life Balance)
- pH probe
- 4 x stirring rods
- 1 mortar + 1 pestle.
- Safety goggles & Lab Coat
- Thermometer
- Pen & Logbook
- Heating Block

reduce amount of acid secretion. 13/6/23

- Gastric acid reduction can occur due to the use of PPI drugs - Proton Pump Inhibitors - which are used for GERD (A symptom of Hypochlorhydria)
- Overuse of antacids to treat the acid reflux symptom can lead to a case of medically-induced Hypochlorhydria.
- Hypochlorhydria symptoms are often mistaken for Hyperchlorhydria.
- Large amounts of Betaine HCL taken can potentially burn the lining of stomach, can also cause nausea.
- > 650mg of Betaine HCL should not taken without a physician's recommendation.
- People with a medical history of gastrointestinal symptoms should take a doctor's consult before consuming Betaine HCL.
- Hypochlorhydria diagnosis & Betaine HCL consumption should consulted with a certified physician.
- Severe hypochlorhydria is "Achlorhydria."
- People who are of the age of 65 and up, who have experienced autoimmune conditions, or have extensively used antacids or PPI's are more likely to show symptoms of Hypochlorhydria and be diagnosed with Hypochlorhydria
- Autoimmune conditions such as autoimmune gastritis - a chronic, inflammatory disease that destroys the parietal cells found in the corpus & fundus section, in the mucosa of the stomach.
- More symptoms of low acid secretion also include cramping & brittle fingernails.
- Aging is the primary cause of low acid secretion.
- Dietary supplements aren't FDA regulated, but → supplement companies have the liability of providing supplements that have some evidence of being safe and the labels carry out intended purpose. As long as product doesn't carry a "new" dietary active or excipient ingredient, the FDA won't remove it.

Pepsin & Other Aiding Ingredients

- Betaine HCL supplement is often provided with pepsin to further aid the effectiveness of the supplement.

- Bitter tasting plants aka "bitters" are known for being commonly used to promote healthy digestion.

- Even though there isn't much pre-recorded info about Betaine HCL, recent studies are showing in fact acting effectiveness when used for meal-time supplementation.
- Clinicians have started recommending Betaine HCL for this exact reason.
- Higher amounts of pepsin + other aiding ingredients can increase the effectiveness of the Betaine HCL supplement.

- Often dietary supplements are combined with digestive enzymes such as pepsin to help with protein digestion.

13/6/23

13/6/23

Research - HCl SUPPLEMENTS

- How do they work?
- What makes them work? → • Purpose of this medication?
  • Outcome of ^(after) consumption which indicates the medication worked?

DOCTOR'S BEST —

Betaine HCl + HCl → ? (aqueous)

$C_5H_{12}ClNO_2$ + HCl → ?

or Betaine HCl + gaqueous lumen

$C_2H_{12}ClNO_2$ + ?

What is the chemical equation to describe reaction? (of re-acidification)

- 650 mg of Betaine HCL
- 250,000 FCC Pepsin Units → ? mg → 1:10000 → 10000 FCC/mg

$\frac{250000}{10000} = 25$ mg?

- 20mg of Gentian (Gentiana lutea) root
  ↳ It's a bitter.

- So, far this supplement brand seems the most promising. as it contains 2 aiding ingredients. But they together only make up 45mg of aiding ingredients.

VITACOST —

- 650 mg of Betaine HCL
- 162 mg of Pepsin.

- This seems more promising as the amount of pepsin per serving/capsule is higher than the other supplement brands.

NUTRICOST —

- 650 mg of Betaine HCL
- 140 mg of Pepsin

- Pretty average, but higher amount of Pepsin compared

Educated Guess

HYPOTHESIS ABOUT EXPERIMENT

For my Hypothesis - I'm assuming that Vitacost will work the most efficiently out of all other brands, as Vitacost contains higher amount of Pepsin per serving/capsule. Which will help the gastric acid, re-acidify faster.

18/6/23 Risks & Hazards

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☐ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: Raajvi Shah ID:

SCHOOL: Our Lady of the Sacred Heart College

Activity: Give a brief outline of what you are planning to do.

This investigations' purpose is to determine which brand of Betaine HCl supplement will best acidify the stomach acid. The lowly acidic stomach acid will be recreated through the use and dilution of hydrochloric acid (pH 0, 1M), each trial will be gently agitated using the same swirling motion made by the hand for the solute to dissolve into the solution. A pH probe will be used to measure the initial pH of the diluted solution and the pH of the solution after the reaction occurs. The brands being tested are Doctor's Best, Nutricost and Vitacost.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
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- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Type of Risk?	What is the risk?	How can I control the risk?
Chemical Risk – Used and Produced Hydrochloric acid (aq) <3 (<10% wt/wt.)	Higher Concentrations (<3 (<10% wt/wt.) if not handled with care, it can causes serious hazards and fatal risks, such as irreversible damage or fatal risks to internal organs such as lungs upon inhalation of vapour, skin, and eyes upon	Avoid inhalation of vapour and follow standard handling procedures as in, do not touch direct, be careful when handling, and avoid experimenting HCl with, store only small amounts. Keep away from other (especially incompatible) materials such

18/6/23

Risks & Hazards

	direct contact, as it is a corrosive acid.	as oxidizing agents, organic materials, metals, and alkalis. HCl can corrode through metal surfaces.
Physical Risk - Water <43.5°C (cold-warm) & >50°C (lukewarm – hot)	Harmful when in direct contact and consumption when >50°C. Harmful when being tested in a laboratory. Generally, water below 43.5°C is considered safe for adults and children, but prolonged exposure to cold water can cause numbness and in worse cases, hypothermia.	Do not drink the water in a laboratory as it could be contaminated with harmful, chemical mist and vapours. Keep safe by not touch the water when it is above 50°C as it will cause major burns. Water spilled on the floor is a slipping hazard.
Chemical Risk - HCl supplements (Doctor's Best, Nutricost, Vitacost)	Consumption without recommendation of certified physician for assumed or not tested diagnosis can cause problems such a burning sensation in the chest area (heartburn). Larger amount consumption can burn the stomach lining.	Do not consume unless you have been diagnosed with Hypochlorhydria, Achlorhydria or have been recommended by a certified physician. Especially do not consume is a laboratory as it now prone to chemical contamination.
Physical Risk – GLASSWARES - Alcohol Thermometer, Beakers (2 x 100ml), Conical Flasks (9 x 250ml), Measuring Cylinders (2 x 100ml) & (1 x 10ml)	If not handling properly, the thermometer will break and risk the experiment conductor and others in the vicinity of being injured by glass fragments	Carefully sweep broken glass and glass fragments with a brush and a dustpan. Do not attempt to use fingers.
Pipettes (5 x 4ml)	If not handled properly the chemical that is filled in the pipette could eject out and	

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Risks & Hazards

	cause the chemical to come in direct contact with yourself and other people surrounding the experiment. Can be confused with other pipettes used which can lead to contamination of chemicals which can lead to unknown reactions.	Make sure to separate used and unused pipettes and handle the pipette with care.
pH electrode (pH probe – glass electrode and pH sensor)	The glass electrode of the pH probe could break which can release the toxic chemicals used to help its function.	If the glass electrode snaps, clean up the fragments using a brush and a dustpan. Do not attempt to pick up glass fragments with your fingers.
Mortar (came with pestle)	When broken can cause deep wounds and cut	Carefully discard of broken pieces.
Electric Hotplate	This is an ignition source, therefore do not let flammable liquids sit atop its surface when turned on and off, when its isn't certified as spark proof. Burns can occur when hot plate is in direct contact with skin whilst the hotplate is turned on and afterwards as the hot plate retains heat. The electrical cord could also get damaged by heat and cause an electric shock	Inspect cord regularly for signs of damage this could be that the cord is loose in the plug, the cord is loose at the entry to hot plate, or that the cord may have signs of corrosion or other damage. The hot plate must be tested and tagged at regular intervals by an adult or a lab technician. Ensure that the hot plate's cord is made of heatproof material. Do not touch hot plate when turned on and after it is turned off.
Electric Kettle	This is ignition source on the inside, no other liquid must be placed in the kettle other than	Inspect cord regularly for signs of damage this could be that the cord is loose in the plug,

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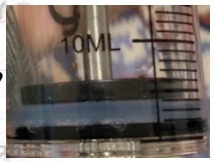
Risks & Hazards

	water, as chemical mists and vapour will be released and inhalation of those is harmful to health. The body of the kettle is still hot after use as the body retains heat from the liquid heated inside earlier. The electrical cord of the kettle could also be damaged by heat caused through kettle and cause an electric shock. Hot water inside the kettle can cause major burns when came in direct contact.	the cord is loose at the entry to kettle, or that the cord may have signs of corrosion or other damage. The kettle must be tested and tagged at regular intervals by an adult or a lab technician. Ensure that the kettle's cord is made of heatproof material. Do not touch kettle by the body, only use the handle when the hot water is still in there and after the water has been disposed of. Do not touch the hot water inside the kettle.
Chemical Risk – Plastic Bulb Pipette (not included in final materials list as it was not used in the last experiment but was used briefly in the 2nd experiment.)	Ingestion can occur if one uses their mouth to fill pipette from the top. Organic solvents may swell the surface layer of plastic, which could causing cracking and leaking of the pipette.	Do not use mouth to fill pipette , always use a properly fit pipette filler to fill pipette. Do not fill pipette with organic solvents and do not rise and clean pipette with organic solvents.

Mock Experiment 19/6/23

- As my inquiry's due date approaches around the corner, and I'm yet to have done even 1 experiment....
- I have taken it upon myself to trial it at home! not with HCl acid! with food colouring!! Yay! :)
- So I am prepared and manage my time better on the day of experiment!
- I will use the method I made 12 days but I will substitute HCl acid with food colouring - and I will substitute the equipment for the closest items I could find in my house.
- However, I had to

Barbecue syringe increments



Items, Materials & Food colouring! –

- The barbecue syringe was used as a pipette
- Spoons & bowls were used as mortar & pestle
- The orange food colouring in the medicine cup was used as 1M HCl acid.
- The glass cup in the top left corner was my first dilution 0.01 concentration.
- The green plate is my pretend hot plate.
- The further diluted solution (0.0001 concentration) is in the cup atop the "hot plate".
- The wooden skewer is my pH probe
- The barbecue thermometer is my thermometer,
- Straw is my glass stirring rod.

Dilutions side by side –

- The small cup on the right-hand side is supposed to represent my HCl acid. (1M)
- The cup on the left hand side is supposed to represent my 1st dilution (0.01M)
- The middle cup represent my last dilution. (0.0001M)

What this means –

That my experiment will go perfectly fine as far as dilutions go! But ~~the~~ this was measured and diluted by 1ml for the initial dilution as in 1ml : 100ml not ~~0.1ml~~ : 100ml so that only differs, but the concentrations in this mock trial are wrong ~~for~~ according to my method. meaning my method will work

21/6/23

Experiment Time!!!!

- The Betaine HCl's supplement when cut open looked ivory white with some translucent crystals present.

28/6/23

- After some research help from my teachers, I have been able to show the chemical equation that supports the purpose of Betaine HCl as a direct supplement in acidifying the stomach

- **Reactants** → **Product**

Betaine HCl (s) + Water → Betaine (aq) + HCl (aq)

(s) = solid

↓ as the pH used for final experiment was 7.2.

as water makes it aqueous! YAY!

Thanks to Mr. Overelle for helping me with this!

pH 6, 20°C → pH 2.4, 20°C

pH 6, 18°C → 21/6/23 → 2.6, 18°C

pH 6, 17°C → pH 2.4, 17°C

21/6/23 NOT THIS THOUGH

Vita Cost supplement also had sediments sitting at the bottom once the solution had time to settle from swirling.

Supplement Brand	1st Testing (pH)	2nd Testing (pH)	3rd Testing (pH)
Doctor's Best	2.4 Temp - 20°C	—	—
Vita Cost	2.6 Temp - 18°C	—	—
NutriCost	2.4 Temp - 17°C	—	—

21/6/23

Experiment Time !!!

- Today at Lunch, on this day, the 21st June - came the day to finally do the experiment in 45 min time period.

- Honestly, I'm really nervous, about how we will go for time, if experiment will work? I sure hope so that my experiment is a success.

POST - EXPERIMENT NOTES, EDITS, ERRORS, MISCALCULATIONS =

- So, we ended up changing half the experiment to due time constraints and incredibly fast approaching deadline -

Step 1 -
measuring out 10 ml of HCl acid (pH-0, 1 M) in a beaker

Step 2 -
measuring out 99.9 ml of distilled water in a measuring cylinder (using pipette if needed to get accurate measurement.)

Step 3 - (bulb)
using a 2nd bulb pipette (just for HCL), 0.1 ml was measured out from 10 ml HCL acid in the beaker, and was dropped in the measuring cylinder.

Step 4 -
Using the same 2nd pipette, we measured out 10 mL of 99.9 mL of distilled water + 0.1 mL of HCL acid and pour that into a conical flask.

Step 5 -
Using a 2nd measuring cylinder, measure out 90 mL of distilled water and that was poured into the conical flask filled with 10 mL of 99.9 distilled water + 0.1 ml of HCL acid (pH-0, 1 M). We measured the pH of this new solution and it turned out to pH - 6 ? (1st error) (not bad error though as a person with Hypochlorhydria will have a varied pH of 4-6, plus on the bright side pH 6 can show a more realistic change from slightly acidic to highly acidic → pH 0-3).

21/6/23

Step 7 -

We then tried to heat up the conical flask on the pre-heated hot plate BUT - That did absolutely nothing! The temperature of the acid before hot plate "heating" (we tried to see temp before with thermometer) was 18°C and about 7 mins after it increased to 20°C! :\ (2nd Error) We were running out of Lunch time as preparing the 99.9ml water + 0.1 ml HCl acid solution took 20 mins alone! (major setback)

Step 8 -

However a minor improvement was that we didn't have to crush the supplement, we simply had to cut the capsule in a mortar so the supplement powder stayed contained. We tested Doctor's Best first

Step 9 -

We then mixed the supplement powder with the diluted acid solution (pH - 6) and swirled the supplement + acid solution by the neck of the flask to still imitate stomach churning. This was done until powder was dissolved

Step 10 -

We rinsed the pH probe again to test the final pH of Doctor's Best + diluted HCl acid solution ... AND IT WORKED! The pH 6 acid solution re-acidified to pH of 2.4!

Step 11 -

We then began testing on Vitacost & Nutricost, however, due to time constraints we weren't able to leave their solutions on the hot plate for long and so Vitacost's solution was 18°C and Nutricost's solution was 17°C! But at least the pH of all solutions were 6 as the testing baseline. (Also, in the Vitacost solution - some sedimenting occurred after) Step 12 - (the solution settled?) (3rd error)

We ran out of time to complete the remaining 2 rounds of testing, meaning I have to do it next Wednesday :\ But this gives me time to figure out some ways to avoid errors and my miscalculation of pH 4 & pH 6.

25/6/23

Figuring Out How To

WORK AROUND MY ERRORS & MISCALCULATIONS.

1st Error - Calculations were meant to give my solution the pH of 4, but instead it gave me the pH of 6? (Serial dilution) { Dilemma }

- But how - through my research source the increments of a 1:10 ratio was used to get pH 3, 4, 5, 6, 7 from HCL acid. The site calculated that if 0.1ml of HCL acid (1M) is added to water until final volume is 100mls, then it will have the pH of 3 - meaning 99.9mls of water. I used this to based my acid solution on as a 100ml solution is easiest to use the ratio on.

- The equation uses 0.1ml HCL acid + 99.9ml of water to get pH 3 HCL acid solution and used the ratio of 1:10 as the constant dilution factor to increase pH by 1. 10mls of 100ml solution should be from intial solution.

- Ratio of HCL acid to distilled water in intial solution is - 0.1:100 → which is 0.1% HCL acid solution (1M)

- 1M HCL gives pH0, 0.1M HCL gives pH 1, 0.01M HCL gives pH 2, 0.001M HCL gives pH 3, 0.0001M HCL gives pH 4, 0.00001 HCL gives pH 5, and 0.000001M HCL gives pH 6.
  ∴ My acid solution is HCL = 1×10^{-6} M but how?

- Well, I used 0.1ml of my actual stock solution, and if 0.1ml is divided by the volume of my overall solution (HCL + water) then I'm left with 0.001 concentration of HCL, which does give me pH 3 √√. Then what went wrong?

- $\frac{x}{100} = 0.000001 \times 100$ → ∴ the solution I mixed up earlier must have equalled pH of 4, and not 3.

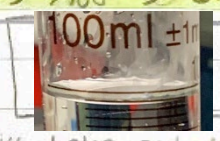
  $x = 0.0001$

Unless I only added a drop of HCl acid, as I remember that 0.1ml was coming out in drops - meaning I assumed 0.1ml came out, when

25/4/23

really only 0.01 ml came out. That means the pH of the initial solution must have been 4 itself, and when further diluted, it became pH 6.

- ~~looking back at the pictures~~, 99 mls of water stayed slightly above the increment line (0.01 ml HCl)
- NOW! as great as it is to get pH 6 accidentally. I can't make pH 6 again, as I won't be able to measure out exactly 0.01 ml of HCl acid again, therefore I'll stick to making my original pH solution level 4, but with a few changes to my measurements A method to assure I can get pH 4 solution when testing my supplements.
- But I was stressed due to time constraints. I failed to notice it.
- I will measure out 1 ml of HCl acid and pour in distilled water until total volume = 100ml. Because 1 : 100 → 1/100 → 0.01, which gives me the pH of 2.



- Then using 1:10 ratio of serial dilution, I will take out 10 ml of pH 2 solution and dilute it further with water until that solution's volume = 100 mls. Because 0.01 : 100 → 0.01/100 → 0.0001 concentration of HCl acid in the solution, giving my solution the pH of 4. (water will be measured using a measuring cylinder.) (10 ml in a conical flask.)

To make sure that my intial solution equals to pH 2, I will measure using a pH probe?

2nd Error - Time! How to fix time management?

- Well, I have been talking to my science coordinators, so hopefully I'm able to arrange a longer time period before due date to submit by project! :) Wish Me Luck!

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3rd Error – solutions temperature? How to make sure all solutions temperatures are same at 37°C or 40°C to make all testings a fair testing.

- My last testings were not a fair test because each solution had different temperatures.
- So, how can I heat up my solution to at least 40°C to imitate the stomach temp when churning & digesting.
- I could also heat up solution to 37°C to accurately match stomach temp, which requires constant monitoring
- I also need to heat up the water I'm using fast, because of time constraints.

Some heating options:

- Water Bath
- Kettle
- Hot Plate (which didn't work last time and needs to be set up 30 mins prior, which it was set up last time)
- Tripod + Bunsen Burner (To risky with acid solution being aloft)
- Or leaving solutions at room temp, as last time they were all around room temp and that didn't affect the effectiveness of the supplement - But higher temp would accurately show the most effectiveness you can get out of a supplement, with no sediments remaining behind.

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- With a kettle, I could heat up the water, then pour some of the heated water in a measuring cylinder, and then pour some room temp distilled water in to cool the temp down to 40°C.

- All of that must have the volume of 90 mls, then I'll pour that into the conical flask with 10 mls of HCL + water solution, (pH-2, 0.01M), which will probably further bring temp down closer to 37°C.

- Or unless the hot plate works, then I could heat the solution up to 40°C, and commence testing from there.
(it won't because my science coordinate can't set it up earlier)
- My kettle plan however seems like a fool proof plan. ☺

- So, we'll try my kettle first!

Experiment time!

RUN #2. 27/6/23

Brands	Test 1	Test 2	Test 3
Doctor's Best	pH 6.7 32°C = pH 2.3 (34°C)	pH. 7.2 32°C = 2.4 (34°C)	
Nutricost	pH 7.2 32°C = 2.3 (53°C)	pH 7.2 32°C = 2.5 (34°C)	
Vitacost	pH 7.2 Temp-32°C ~~pH~~ 2.4 (33°C)		

Sediment Problems ✓ Lost Materials

10:44 - 40°C reached
10.54 - 38°C reached
heated water temp.

- Today was much better than last time, however I'm still confused as to why how the pH went higher for the calculations I edited, where it should have been pH 4 solution before - supplementation.

→ Discussed more after next page.

27/6/23

New Method to Follow

1. Set up the supplements in front of a mortar & set up 3 conical flasks in front of each mortar.

2. Measure out 5 ml of HCL acid (pH-0, 1M) in a beaker.

3. Measure out 99 ml of distilled water in a measuring cylinder.

4. Using volumetric pipette, measure out 1 ml of HCl acid from beaker and drop 99 ml of distilled water.

5. Using a pH probe, measure the 100 ml solution of HCL + water's pH, it should be 2.

Step 8 after steps 6 & 7 as shown.

8. Use a clean volumetric pipette and measure of 10 ml of pH 2 solution and drop into each conical flask (10 ml × 9 flasks = 90 ml of pH 2 solution)

6. Measure out (9 flasks × 90 ml of distilled water = 810ml) 810 ml of distilled water & heat up in a kettle.

7. (Record amount of heated mls of distilled water plus amount of mls of room temp distilled water needed to make this solution) (have temp of 40°C)

9. 7.

9. Measure initial pH of this solution using a pH probe (should be pH-4)

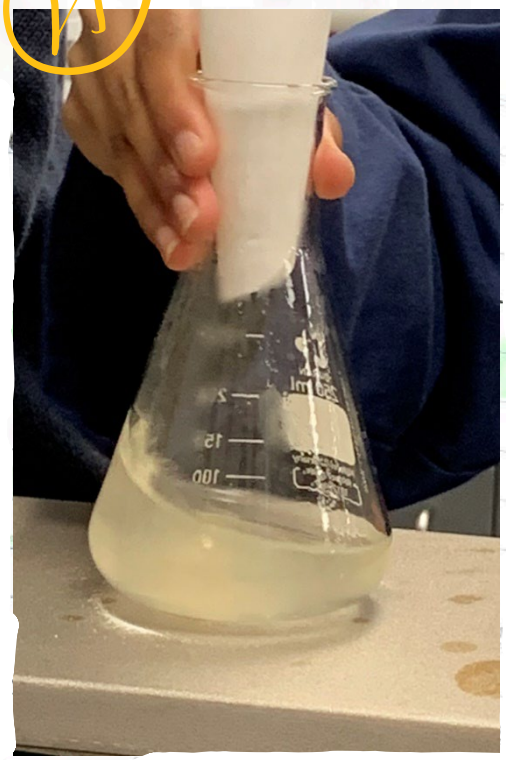
10. Cut open a supplement tablet in its assigned mortar, and swirl the tablet in first conical flask filled with pH 4 solution (Temp-40°C)

11. Swirl until dissolved .10

12. Record new pH using a pH probe. .11 .12

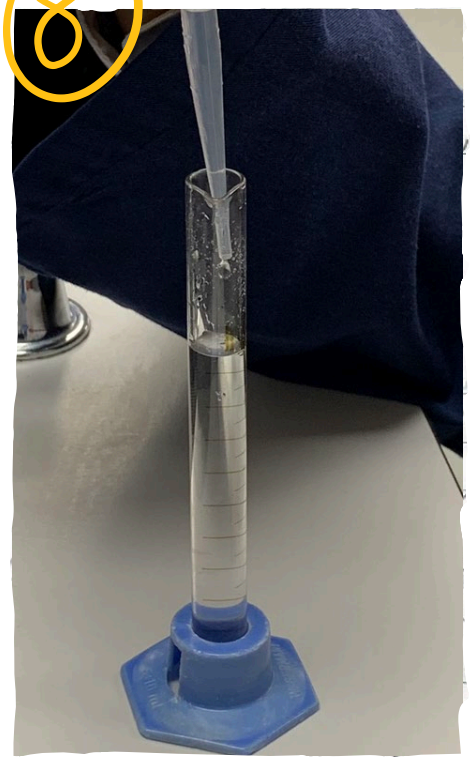
1

11

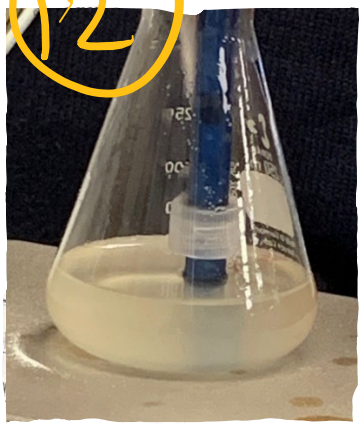


2

8



12



13. Repeat steps 8-12 x2 on the same supplement / brand.

14. Repeat steps 8-13 on all other supplement brands.

Today's Experiment

27/6/23

SETBACKS, IMPROVEMENTS & MISCALCULATIONS.

- Today's experiment worked out better, however there are still some minor setbacks & miscalculations, I want to clarify before I finish my experiment tomorrow at lunchtime.

- The bulb pipette (volumetric pipette) failed me. ∴ Because the bulb blower part got filled with some of the solution, and that wasted 15 mins! And it dribbled is messy drops in the first conical flask.
- That first setback messed up first solution in the testing, which was the Doctor's Best supplement. Therefore, a slightly more amount of the pH "2" solution was added to that first conical flask - Hence why Doctor's Best's first testing's intial pH was 6.7 unlike the others 7.2.
- We didn't have enough distilled water to heat up in the kettle, so we had to use tap water.

Second setback could have overcame if I had just tipped out the small amount of pH "2" solution and restarted that 1st conical flask.

- How did the pH "2" solution turn into pH 7.2 once diluted again! I thought my plan & calculations were foolproof -

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but I guess I got humbled really quickly. I understand what happened to Doctors Best first testing, but the others! HOW!

- I had measured out exactly 1 ml to drop into exactly 99 ml of distilled water - which should have given me a 1:100 ratio → which should have been 0.01% of concentration → pH 2!

- Then I had (except 1st testing of Doctor's Best) exactly 10 ml of that first solution - (pH 2) - and poured in 90 ml of heated tap water and the entire solution together had the pH of 7.2! and temp of 32°C!

- Therefore the calculations for this concentration should have been 0.01×10:100 → 0.1:100 → which actually would have been wrong to my calculations and should have been pH 3, concentration = 0.001

- But it ended up as pH 7.2?! Which neutral?! Could it be the tap water use?! Temperature change?!

- This means I have tested to increase acidity in those who have ACHLORHYDRIA! (don't produce or secrete any HCl acid at all!)

- But to make my experiment a fair test I must my same (wrong for pH 4) calculations to make sure pH is 7.2 again.

- The temperature was also meant to be 37°C celcius to imitate stomach temp when digesting - but it turned out to be 32°C before testing.

27/6/23

- Maybe because the heated water reduced by 2°C (40°C - 38°C) whilst I finished prepping the flasks with 10 mls of my pH "2" solution - and then I poured 90 mls of that into a measuring cylinder to make sure I got 90 ml, and then mixed it with the 10 ml of pH 2 solution, which was at room temperature.

- As I am running very quickly I can't make anymore measurement changes to my calculations for temperature & pH, and I still need to complete my 5 remaining testings - 2 for Vitacost, 1 for Nutricost & 2 for Doctor's Best to account for the 1st messed up one. ☺

To make sure my experiment is a fair test - I'll have to go along with the measurements to make pH 7.2 and have the temperature of 32°C.

- Heated water was at 40°C because my teacher and I poured a little out at a time, then poured a little amount of room temp tap water back in to substitute for quantity loss and to decrease the temperature.

- So I can only hope, manifest & pray that tomorrow my remaining testings run smoothly, and that I measure exactly as I did today (for testings 2, 3, 4, 5) so that the pH is 7.2 & the temperature before reaction is 32°C.

- Also, I noticed that after the reaction that the temperature of the solution had increased in all testings - however, it is unclear as if the temperature was caused by the hot plate underneath or by the reaction, as I put a hot plate underneath to maintain temp of 32°C before reaction to make sure it's a fair test.

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- But it can also be noted that during reaction the conical flask was raised above platform to imitate stomach churning, by gently swirling the solute & solution. And after solute was thought to have been dissolved was, when flask was set back down to measure new pH & temp.
- The sedimentation at the bottom reduced due increase in temp (which was dissolving more) powder
- I will also clarify with my teachers if I can substitute a new, more accurate testing for the 1st testing of Doctor's Best, so my results can be more accurate and my experiment can be a fair test. ☺
- Some material was lost during experiment as we didn't have a spoon to scrape out material from mortar.

Experiment Time!!!!

RUN #3 - THE LAST RUN!!!!

28/6/23

2:4 7.2 28/6/23 → was done after Vitacost #2

Brands	Test 1	Test 2	Test 3
Doctors Best	pH - (°C -) → pH (38°C)	pH - 7.2 (°C - 32°C) → pH 2.4 (34°C)	pH - (°C) → pH ( )
NutriCost	pH - 7.2 (°C - 32°C) → pH 2.3 (33°C)	pH - 7.2 (°C - 32°C) → pH 2.5 (34°C)	pH - (°C) → pH ( )
Vita Cost	pH - 7.2 (°C - 32°C) → pH 2.4 (33°C)	pH - 7.2 (°C -) → pH 2.4 (40°C)	pH - (°C) → pH ( )

2.3 39°C

New Method & Materials List ☺☺☺

(3 trials per supplement)

1. Start by boiling water (Amount = 1.25 L) in a kettle & set up hot plate.
2. Set 1 mortar & 3 conical flasks to each supplement brand.
3. Measure out 10 ml of HCL acid (ph - 0, 1 M) in a beaker
5. Measure out 99 ml of distilled water in a measuring cylinder. Use a pipette to get more accurate results.
4. Break a capsule of the corresponding supplement brand in the mortar.

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6. Using a volumetric pipette measure out 1 ml of HCL acid and drop into 99ml of distilled water's measuring cylinder
7. If kettle is done by now - then begin pour out small amounts & substitute those amounts for room temp water - to lower temp to 40°C.
8. Once temp is lowered to 40°C shut the kettle lid & leave it for 10 mins - this will bring temp to 38°C.
10. During those 10 mins, measure out 10 ml of 1m HCl acid (1M) + 99 ml distilled water, using a clean pipette, and drop into each conical flasks (meaning 10x9 = 90 ml)
11. Once temp of heated water has lowered to 38°C - begin by measuring out 90 ml of heated water, use a clean pipette to get more accurate results. Measure in a measuring cylinder
12. Once measured to 90 ml of heated water in a measuring cylinder, set conical flask on the hot plate and carefully pour in the 90 mls of heated water.
13. Record the pH and temp for the solution using a pH probe and a thermometer.
14. Use a clean sheet of filter paper over the conical flask as a funnel and tap the supplement powder from the mortar.
15. Swirl around solute & solution together by holding flask by the neck and swirl until solute is dissolved.
16. Set solute + solution conical flask back down on the hot plate and measure new pH & temp (clean pH probe and thermometer with each trial.)
17. Repeat steps 4 & 12-16 with the same supplement brand 2 more times
18. Repeat steps 4 & 12-17 with the 2 other supplement brand.

28/6/23
~~I can't graph it sadly~~ as I was running way out of time, so I couldn't measure I was pouring out and substituting room temp water back in.

using pipette to take out small amount add back in same small amount YAY!

might have to go with approx. measuring

But might not have enough

99°C 89°C 75°C 63°C 49°C 46°C 40°C

- That is not my final & polished method. Will polish it in report.
- I should simplify method to each trial - not the one I'm doing to speed the process up.

28/6/23

Materials List

- 9 x conical flasks
- 3 x mortars
- 1 x pH probe
- 2 x 100 ml distilled water bottles
- 1 x kettle
- 1 x hot plate
- 3 different supplement brands
- 4 x pipettes ( ) ← capacity 2
- 1 x volumetric pipette ( )
- 9 x filter papers
- 1 x thermometer
- 2 x beakers
- 2 x measuring cylinders
- 1 x Laptop
- 10 ml of HCl acid (pH 0, 1 M)
- 800 ml of tap water
- 1 x Logbook
- 1 x pen
- 1 x lab coat
- 1 x safety goggles.

Pre - Experiment Thoughts

- I hope today I can recreate yesterday's pH level & temperature! ☺

Post - Experiment Thoughts

- It almost worked... ☹. I ran out of time to complete my last round of trials ☹, but I had all pH's matched, but the temperatures didn't correspond to yesterday's!

29/6/23

Analysis of Results

- Experiment was not reliable due to temperature fluctuations in the 1st trial of Doctor's Best (which was re-done due to the pH being 6.7 and 7.2) & 2nd trial of Vitacost (which hadn't been done 2 days ago and I needed to get at least one more done for Vitacost. The temperature on the 27/6/23 were 32°C, but on the 28/6/23 they were 37°C - 39°C. Hence why it's an unfair test.
- I ended up testing Achlorhydria. (Neutral pH) not Hypochlorhydria (mildly acidic)
- My practical was heavily time constrained and time left wasn't sufficient for conducting a 3rd rounds of trial for each supplement. Had there been enough time, and my results would have been more reliable & accurate.

- An average can be made on 2 testings but 3-5 testings' worth of average for a supplement would have been more reliable.
- This is something I hadn't realised before but it makes sense as I ordered supplements with same amounts of Betaine HCL.
- One thing I noticed was the pH wasn't dependent on the temperature! Meaning that the pH stayed consistent despite temperatures being different, so my results might be slightly valid after all.

With further research & teacher clarification: Pepsin was not involved in the pH change as it is only a catalytic enzyme.

E.g → Doctor's Best Trial 1 (28/6/23) = pH 7.2 (37°C) → pH 2.4 (38°C)
Doctor's Best Trial 2 (27/6/23) = pH 7.2 (32°C) → pH 2.4 (34°C)

meaning more trials need to be done for research to see how chemically different Vitacost is.

- This means my results are not valid and I can prove my hypothesis was correct - (Vitacost is most effective supplement.
Because -
Vitacost = (650mg) Betaine HCl (162mg) Pepsin
Nutricost = (650mg) Betaine HCl (140mg) Pepsin
Doctor's Best = (650mg) Betaine HCl (250 000 FCC units) Pepsin

coming to a final verdict would be biased

meaning that the pepsin concentrations were also being tested. (which is why most results were similar.)

- This proves that Pepsin doesn't only aid in protein breakdown but also aids in increasing acidity.

29/6/23

Analysis of Results

DISCUSSION

- Systematic Errors - errors made by humans in the method, that affect accuracy of results.

→ First of recognise this, experiment must be conducted multiple times, so I have included the experiment conducted on the 21/6/23 to help with the recognition of systematic errors.

- The pH calculations were always incorrect - as, on the 21/6/23 the pH was consistently 6 instead of 4, and 27/6/23 - 28/6/23 the pH was consistently 7.2 despite increasing the HCl acid acid concentration from 0.1ml to 1ml in the first serial dilution

NEXT TIME → Can be improved with further reseach and testings to see which series of dilutions can give the pH of 4 for intial solution before acidification.

- The temperatures were always inaccurate due to there not being a consistent method to always achieve the same temperature, Hence why the temperatures on 27/6/23 was different to the temperatures on 28/6/23 A and the temperatures on the 21/6/23.

NEXT TIME → Can be improved by having ~~more~~ going further into detail with amount of heated water poured out, as that can show consistent decrease in temp each time consistent amount of heated water is poured OUT & substituted with normal temp water. → until its 37°C → that way ~~stomach~~ Stomach temp of 37°C is recreatable and repeatable

Further Improvements

Instead, further improvements should include, timing each supplement brand after the reaction occurs as, that will tell us more about it's effectiveness - it's about how much it can change acidity & how long can it sustain it. → that will provide greater accuracy

- Conducting more ~~tests~~ trials per brand
- Further reseach and calculations for pH 4 solution testing.
- Further improve method by adding more detailed temp calculation for achieve temp of stomach acid with ~~a~~ repeatable steps- e.g. 25 ml of heat water has disposed off and replaced with normal temp water 5 times until temp

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Thank You! from me,

Raajvi Shah.