Investigating The Expectorant Properties Of Different Cough Remedies On The Treatment Of Cough

Research Question:

To what extent do different cough solutions (Ginger, Robitussin PS and Robitussin lozenges) vary in influence over viscosity of artificial mucus?

Subject Area: Biology

Word Count: 3992

Exam Session: May 2020

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1.0 Research Question

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2.0 Introduction

A cough is a common symptom, part of many diseases, and occurs through the airway. A cough is used to clear the throat of the pathogens and irritants that may be present in the airway lining. Mucus gathers these particles together, and cilia helps to move the mucus through the lining, up to our throat, to discard it from our mouth or nose. The uncomfortable feeling one gets that induces a cough is created through the cilia. This process prevents infections, helps relieve any itchiness you may have in your throat and make your day more comfortable.

Robitussin PS is a cough medicine with the active ingredient, *Guaifenesin*. This is an expectorant; it helps thin mucus, to make it easier for the mucus to move through our airway. Robitussin DM contains *Guaifenesin*, however, also contains an antitussive known as *Dextromethorphan*. This is a cough suppressant that assimilates to the brain to block the cough reflex *(WebMD, 2019a)*. However, in terms of obtaining Robitussin DM, the Health Products Regulation in Singapore prevents people from having more than 15 mg of *Dextromethorphan* per 5 mL, or more than 240 mL of it in a liquid form, without an approval *(HSA, 2019)*. This is because larger amounts of *Dextromethorphan* can be used as opioids.

These medicines are still unaffordable by many around the world. These people have to depend on home remedies to help relieve their symptoms. Ginger is referred to as a superfood *(Lifeway, 2015)*, for its many benefits. It is high in antioxidants, has anti-inflammatory properties and can help alleviate nausea. Ginger contains *Guaiacol*, which is thought to have disinfectant properties and used as an expectorant *(Drugbank, 2019)*, and thus should be effective in relieving a cough.

So, which remedy is better?

This experiment uses the ball bearing experiment to determine how different cough remedies effect mucus. It can be predicted that the longer the ball takes to travel through the mixture, the higher the viscosity will be. The velocity found through the ball bearing experiment can be used in the Stoke's law equation to find the viscosity.

The resources used were carefully evaluated for their currency, relevance, authority, accuracy and purpose. Governmental websites such as the Health Sciences Authorities, informational health blogs such as Lifeway, websites that are nationally funded by multiple health institutes such as drugbank, and well-known, updated health forums such as WebMD are a few examples of such resources.

3.0 Background Information

3.1 Artificial Mucus

Human fluids are not allowed for use, as stated in the International Baccalaureate ethics guidelines. Thus, artificial mucus had to made.

As stated in a study done by P.K Singh et al. (2002), artificial mucus consists of egg whites as they provide mucoproteins such as lysosomes. The enzymes being used in this experiment were 1% amylase, 1% trypsin and 1% bacterial protease, as these are common enzymes found in the trachea. These contain glycoproteins and act as immunoglobins. Water provides vital inorganic salts present in mucus and help made the mucus more liquid. Therefore, tap water was used instead of distilled water. By not using too many ingredients, it allows the simplification of the mucus, meaning it is easier study the effects of ginger. The normal pH of tracheal mucus is an average of 7.0 pH, and the artificial mucus was adapted to this. The concentration of each ingredient is included in the preliminary findings (page 6). Another component that could be added was mucin. This is a known component present and produced in mucus, however the price was above the budget available (358 SDG for 100g on *Sigma Aldrich (2019)*). Furthermore, mucin could only be obtained through porcine stomach (*Sigma Aldrich, 2019)*, which is allowed for an IB experiment.

3.2 Cough Medicine

(WebMD, 2019b) There are 2 main types of cough medicine:

- Expectorants
- Suppressants

Expectorants aid in the thinning mucus. Mostly, the components of mucus thinned are any organic salts or water. By thinning mucus, the density is expected to decrease. This will ease the movement of mucus and irritants from the airway to the nostrils or mouth, to get rid of the pathogens or irritants present in the throat.

Suppressants (Also referred as antitussives) assimilate to the brain and help increase the irritation threshold in the airway. Thus, the need to cough decreases, and the cough can be suppressed for a certain amount of time.

Please refer to appendix A for the structure comparisons of the different cough remedies.

4.0 Hypothesis

The null hypothesis is that, all methods of cough relief will have the same effect on the artificial mucus.

The hypothesis is Robitussin PS will be a better expectorant compared to the rest of the substances. The other remedies will have a smaller effect on the artificial mucus, as there would be a lower amount of expectorant included in them. The ginger and hot water mixture will be much more effective than the ginger and room temperature water, as the boiling water and ginger mixture is what is normally used in homes. Though these substances can help thin mucus (act as an expectorant), they will not be able to act as a suppressant.

5.0 Method

5.1 Preliminary Findings

The test for viscosity was done using a 5 mm ball bearing. First the ball was dropped through a 100 mL measuring cylinder, but this distance was too little to assume terminal velocity was achieved. As this is necessary for Stoke's law, a 50 mL burette, which had a length of 66.5 cm (±0.1) was used instead. Therefore a 69 mL mixture was made, so there would be enough of the mixture to place in the 50 mL tube.

To decide between artificial albumen and albumen from egg whites, the consistency was considered. The albumen was more homogenous, yet its viscosity was very low, while the egg white was much more viscous but was not as homogenous. Egg whites were chosen to allow easier reading of the ball when it was falling through the burette. Furthermore, egg whites contain mucoproteins that are also present in mucus.

The first try consisted of 150 mL of tap water, 2.5 mL of 1% amylase, trypsin and bacterial protease and 50 mL of egg whites. However, the viscosity of this solution seemed to be too little to be tested on, as the ball travelled through it too quickly. The mixture was also shaken too vigorously so there were too many bubbles present. The mixture was left for 15 minutes and then tested with the ball bearing experiment. It was obvious that there was an excess of water used in this trial. Even after slowing down the video, the ball was still difficult to pinpoint.

The next try consisted of 85 mL of egg whites, 25 mL of tap water and 7.5 mL of each 1% concentration of enzyme with a buffer of 7.2 pH. Though a lower volume of water and a higher volume of artificial mucus was used, the mixture was still too runny. The ball was left for 15 minutes and then tested again. The ball still couldn't be clearly seen in the footage.

The next attempt consisted of 100 mL of egg whites, 15 mL of water, 7.5 of each 1% concentration enzyme and the pH was compromised to be 7.7 pH so there could be a higher viscosity. With this mixture, the mucus was viscous enough to notice the ball in the footage, so this mixture was set as the mixture for the artificial mucus.

5.2 Independent Variable

The variable that is changed is the solution being tested on. The cough remedy added was different in each solution. Here are the different solutions that were made:

Mixture 1- Artificial Mucus Mixture (no cough remedy added)

Mixture 2- Artificial Mucus Mixture + Robitussin PS Syrup

Mixture 3- Artificial Mucus Mixture + 1st Ginger mixture (10.039g (±0.001) of crushed up ginger left in 25 mL of room temperature water)

Mixture 4- Artificial Mucus Mixture + 2nd Ginger mixture (10.079g (±0.001) of crushed up ginger left in 25 mL of boiling temperature water and left to cool down to room temperature)

Mixture 5- Artificial Mucus Mixture + Robitussin Lozenges (5.020g (±0.001) crushed up and added to 25 mL distilled water)

Mixture 6 (negative control)- Artificial Mucus Mixture + distilled water

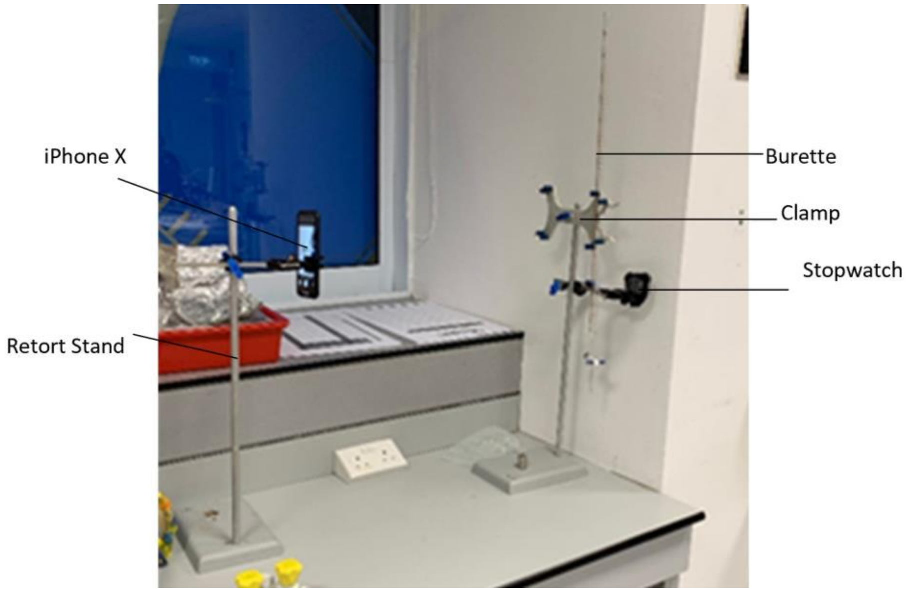
5.3 Dependent Variable

- The measured variable is the amount of time (in seconds) it takes for the metal ball to travel 66.5 cm through the liquid. The ball dropping through the burette was videoed and then slowed it down to find the time when the ball was at the 0 cm^3 mark and the 50 cm^3 mark.
- The derived variable is Viscosity (μ) which is calculated using the time found from the measured variable and Stoke's law.

5.4 Control Variable

The variable that is controlled	Why is this variable controlled?	How is this variable controlled?
The phone which was used to record the ball bearing experiment was the same and the level at all times and the time was measured when the bottom of the ball was at the line.	To avoid parallax error.	The same iPhone X was used to record every video, and the height of the phone was kept constant too. The same video editor (Kapwing) and same scale factor was used on the video.
The same ball was used the entire time.	To make sure that the same density of the ball could be used to help find the viscosity of the liquid.	The same 5 mm diameter metal ball was used throughout the experiment and kept with the equipment.
The pH of the artificial mucus was kept constant at 7.7 pH.	The pH of tracheal mucus is neutral; thus, it is important to keep the pH around 7 to reenact that environment.	A pH buffer and pH probe were used to achieve a pH of around 7.2 to 7.8.
The time that the mixtures were left for was constant at 20 minutes.	To make sure that the mixtures have an equal amount of time to react.	The beaker was covered and kept in a lab at 24 ^{o}C with a stopwatch for 20 minutes.
The volume of artificial mucus mixture was 46 mL and the volume of the other liquid was 23 mL, hence the proportion was 2:1 respectively.	To ensure that an equal amount of the cough remedy is added an equal amount of mucus.	25 and 50 mL measuring cylinders were used to measure each liquid added.

5.5 Experiment



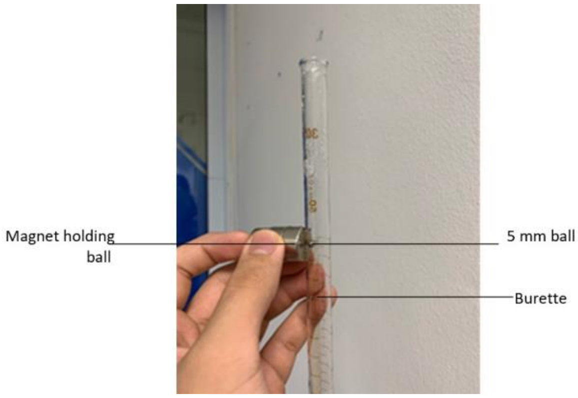
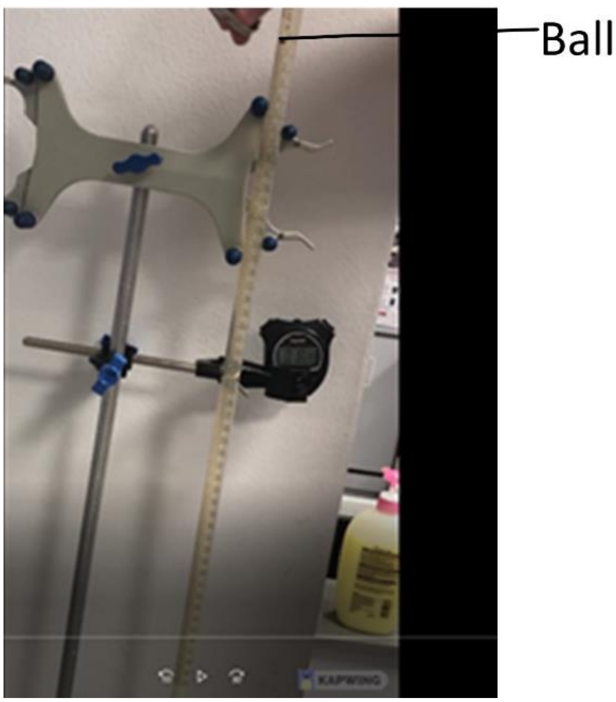


Figure 7a,7b: Images showing the setup for the ball bearing experiment

5.6 Risk Assessment

- Artificial mucus included egg whites (100 mL), tap water (15 mL), enzymes (amylase, trypsin and bacterial protease) (7.5 mL each) and a pH 7 buffer to keep the solution at 7.7pH. 69 mL of artificial mucus was used to experiment the viscosity of mucus. 46 mL of artificial mucus was used in each mixture the components used in mucus were not harmful, though the mixture may taste unpleasant. *(Lillehoj and Kim, 2002)*.

- 23 mL Robitussin PS includes 460 mg of *Guaifenesin* and 138 mg of *Pseudoephedrine Hydrochloride*. The maximum amount of Robitussin PS one can intake is 10 mL every 6 hours. This means if someone were to digest the Robitussin in the measuring cylinder or in the mixture, they would have to consult a doctor.
- 10.079 g of ginger was used in the 2^{nd} mixture. As this was mixed with boiling temperature water. If one were to drink this mixture, they may risk burning their throat or mouth *(Villines, 2019)*. 23 mL of the ginger mixture was used in each mixture with mucus.
- 4 Robitussin Lozenges (5.020 g) were crushed using a hammer and plastic bag. There were 2.4 mg of *Amylmetacrseol* and 4.8 mg of *2,4 Dichlorobenzyl Alcohol (Robitussin, 2015b)*. The recommended number of lozenges is 3, thus if one were to drink the mixture, they would have to consult a doctor.
- To prevent people from ingesting the mixtures, the solutions were kept in a cupboard in the lab, where other students were prohibited from entering.
- If any of the solutions were to spill, the solution has to be cleaned thoroughly by tissue so no one can slip over them. To dispose of the chemicals, they have to be washed down the sink, and the container must be cleaned thoroughly.
- As eggs were used, there was a chance of them rotting if they were left out were too long. Thus, the artificial mucus mixture was made separately every time the experiment was conducted.
- Made sure the hand holding the bag is kept away from the hammer to prevent from harm when crushing the Robitussin lozenges.
- There were no sharp objects, moving parts, ignition parts, radiating particles, electrical parts or eye-damaging parts involved. There are objects than can fall and break. Ways to prevent this are in the equipment list (Appendix B).



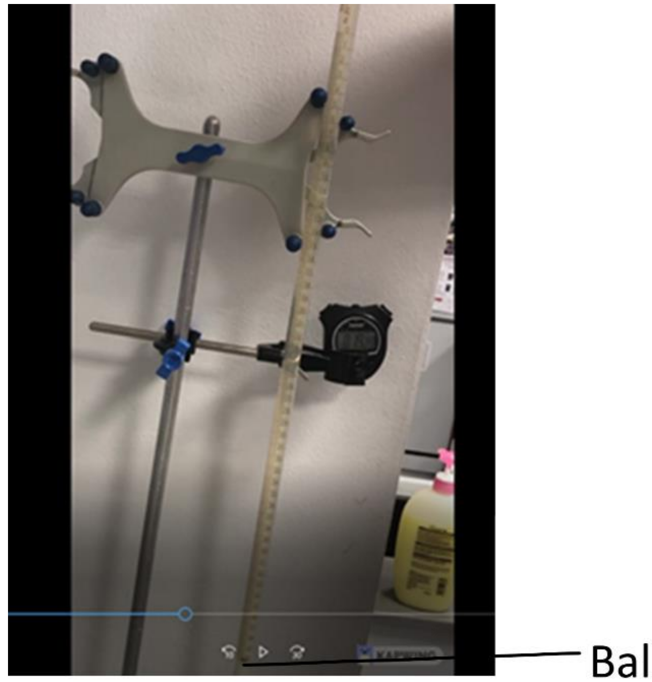


Figure 10: Ball bearing at the top, time at 18.29 s (±0.05)

Figure 11: Ball bearing at the bottom, time at 19.29s (±0.05)

5.7 Procedures

Refer to Appendix B for the equipment list.

5.7a Procedure to Produce Artificial Mucus

1. Crack 5-6 eggs, separating the albumen and the yolks.
2. Measure 100 mL of the albumen using a 100 mL measuring cylinder and place it into a beaker labelled “Artificial Mucus”.
3. Measure 15 mL of tap water using a 25 mL measuring cylinder and place it into the same beaker.
4. Measure 7.5 mL of the three enzymes (amylase, trypsin, bacterial protease) using a 10 mL measuring cylinder and place it into the same beaker.
5. Use a pH probe and a pH buffer of 7 to bring the pH to a range of 7.2-7.8.

5.7b Procedure of Producing the 1st and 2nd ginger mixture

1. Use a mass balance to measure 10.0 g of ginger and place it in a beaker.
2. Measure 25 mL of:

1st ginger mixture- room temperature (24 ºC)

2nd ginger mixture- boiling temperature (100 ºC)

distilled water using a 25 mL measuring cylinder and place it in the beaker.

3. Leave the mixture for 20 minutes.

5.7c Procedure of Producing the Robitussin Lozenges mixture

1. Place 5-6 Robitussin Lozenges bought from a convenience store in a plastic Ziploc bag.
2. Wear safety goggles and use a hammer to crush the lozenges to increase the surface area.
3. Measure 5.02 g of lozenges using a mass balance and place them into a beaker.
4. Measure 25 mL of room temperature distilled water using a 25 mL measuring cylinder and place it into the beaker.
5. Leave the mixture for 20 minutes.

5.7d Procedure of Producing main mixtures

1. Measure 23 mL of a different cough remedy depending on the mixture, as stated in the independent variable.
2. Measure 46 mL of Artificial Mucus using a 50 mL measuring cylinder and place it into a beaker.
3. Leave the mixture for 20 minutes.
4. Repeat the procedure for producing the next mixture.

5.7e Procedure of Ball-Bearing experiment

1. Measure 60 mL of a mixture using a 100 mL measuring cylinder.

2. Place a funnel on top of a burette and pour the mixture until the bottom of the meniscus reaches the 0 mL mark on the burette.
3. Place a recording device of your choice (iPhone X used in this experiment) onto a clamp stand and press the record button.
4. Place a stopwatch onto the same clamp stand as the burette, to make sure the stopwatch can be viewed in the recorded footage.
5. Place the 5 mm ball into the burette with the bottom of the ball at the 0 mL mark using a magnet.
6. Let go of the magnet with no object blocking the view of the recording.
7. Use a video editing app (Kapwing was used for this experiment), slow the video down (as shown in *figure 4*) and note down the timings at 0 cm^3 and 50 cm^3 (as shown in *figure 5 and 6*).
8. Repeat the procedure for each mixture.

6.0 Raw Data

Solution	Initial Time (s) (±0.05)										Initial Time (s) (±0.05)									
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R10**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R10**
Mixture 1	11.29	27.19	40.89	55.69	76.29	10.69	25.89	34.99	49.79	64.29	12.39	28.19	41.89	56.69	77.39	11.69	26.99	36.09	50.79	65.39
Mixture 2	5.29	19.19	35.89	49.09	70.09	7.39	19.09	26.79	38.09	46.79	6.09	20.09	36.69	49.99	70.89	8.19	19.99	27.59	38.79	47.69
Mixture 3	14.58	25.98	45.48	60.08	78.98	8.96	17.86	29.06	43.76	56.06	15.38	26.88	46.28	60.98	79.78	9.86	18.76	29.96	44.56	56.86
Mixture 4	10.49	23.09	32.19	42.19	56.99	65.89	78.19	87.29	98.89	108.59	11.49	23.99	33.19	43.09	57.99	66.89	79.09	88.19	99.79	109.59
Mixture 5	10.59	21.98	34.18	44.68	57.98	67.08	79.08	92.28	104.38	116.78	11.49	22.88	35.08	45.78	58.88	68.18	80.18	93.18	105.28	117.68
Mixture 6	18.29	23.89	36.79	49.09	64.09	4.69	16.79	29.99	42.19	56.39	19.29	24.99	37.79	50.09	65.09	5.69	17.69	31.09	43.19	57.49

Table 2a and 2b: Tables to show the Initial and Final timings for the ball bearing experiment for different solutions with 10 repeats for each solution

The uncertainty of timing is 0.05s, which is more than the uncertainty of a stopwatch, as when the video was slowed down, the stopwatch’s time intervals were 0.1s. Table 2 shows the time of the stopwatch when the ball is at 0 cm^3 on the burette (initial timing (s)) and the time of the stopwatch when the ball is at 50 cm^3 on the burette (final timing (s)). Through this it is possible to calculate the time taken (s) for the ball to travel 66.5 cm (±0.1). Some weaknesses in this procedure were that when the video was slowed down, the stopwatch only showed in 0.10 s intervals which made the data not slightly more inaccurate.

7.0 Qualitative Data

The Robitussin syrup was seen to be denser than the mucus.

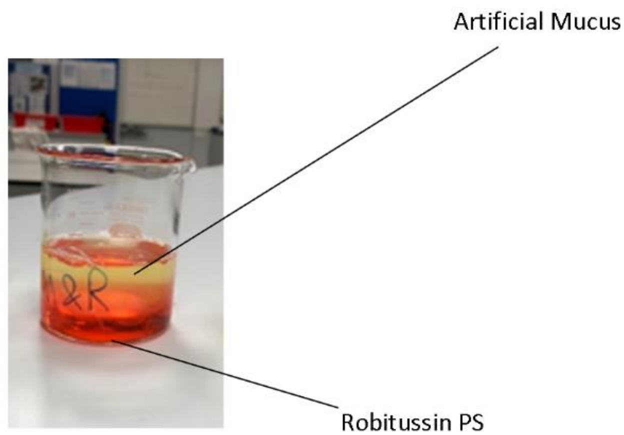


Figure 12: Robitussin shown to move below mucus in the beaker

It could have been assumed that adding a denser medium to the mucus would increase viscosity, as there would be a larger amount of particles/mL that the ball would have to travel through. However, as shown by Table 3 (page 17), the viscosity decreases. A reason for this can be because the Robitussin and mucus were left for 20 minutes, and a solid that floated to the top of the solution was created.

Figure 13: The solid made from the reaction between Robitussin and Mucus

This is red, presumably, because of the colour of Robitussin being red.

This occurred for every reaction except for the control and Robitussin lozenges.

Figure 14: The solid made from the reaction between the first ginger mixture and Mucus, taken by me

As the solids floated, it is assumed that they were less dense than the actual mixture. So, in an actual body, the solid could be easily removed, which would in turn make the mucus less dense and, hence, easier to move up the trachea/airway.

8.0 Processed Data

Refer to Appendix C for the calculations for this experiment.

Time taken for ball to drop through 66.5 cm (±0.1) of solution (s) (±0.10)											
Solution	**Repeat 1**	**Repeat 2**	**Repeat 3**	**Repeat 4**	**Repeat 5**	**Repeat 6**	**Repeat 7**	**Repeat 8**	**Repeat 9**	**Repeat 10**	**Mean (±1.0)**
Mixture 1	1.10	1.00	1.00	1.00	1.10	1.00	1.10	1.10	1.00	1.10	1.05
Mixture 2	0.80	0.90	0.80	0.90	0.80	0.80	0.90	0.80	0.70	0.90	0.83
Mixture 3	0.80	0.90	0.80	0.90	0.80	0.90	0.90	0.90	0.80	0.80	0.85
Mixture 4	1.00	0.90	1.00	0.90	1.00	1.00	0.90	0.90	0.90	1.00	0.95
Mixture 5	0.90	0.90	0.90	1.10	0.90	1.10	1.10	0.90	0.90	0.90	0.96
Mixture 6	1.00	1.10	1.00	1.00	1.00	1.00	0.90	1.10	1.00	1.10	1.02

Table 3: Table to show the 10 repeats for the time taken (final time – initial time) for the ball to travel 66.5 cm through each solution

The uncertainty of 0.10s is calculated as the initial and final timings both have an uncertainty of 0.05, thus when the difference between the two is found, by adding 0.05s with 0.05s. The uncertainty for the mean time of the ball to travel through the mucus is 10 multiplied by 0.1. Thus, the uncertainty is 1.0s.

Example Equation for Density of Mucus:

$$Density\ (gmL^{-1}) = \frac{50.156\ (g)}{50\ (mL)}$$

$$Density = 1003.12\ gmL^{-1}$$

Solution	Mass (g) of 50 mL of the medium (±0.001)	Density (ρ)of mediums (gmL^{-1}) (±0.501)
Mixture 1	50.156	1003.1
Mixture 2	51.605	1032.1
Mixture 3	50.359	1007.2
Mixture 4	50.121	1002.4
Mixture 5	50.795	1015.9
Mixture 6	49.283	985.66

Table 4: Table to show the mass and density for each solution

The uncertainty of the mass is 0.001g and the uncertainty of a 50 mL measuring cylinder is 0.5 mL. Adding these two together gives the uncertainty of 0.501 gmL^{-1}.

Example Equation for Velocity (v) of repeat 1 of Mucus:

$$Velocity\ (v)(cmsec^{-1}) = \frac{66.5\ (cm)}{1.10\ (sec)}$$

$Velocity = 60.4545\ cmsec^{-1}$ (±0.2)

The uncertainty of the distance is 0.1 cm and the uncertainty of the time taken is 0.1 s. Summing this gives the uncertainty of velocity which is 0.2 $cmsec^{-1}$.

Example Equation for Viscosity of repeat 1 of Mucus:

$$Viscosity\ (\mu)(gcm^{-1}sec^{-1}) = \frac{2 \times 981 \times 0.25^2 \times (4048.9 - 1003.1)}{9 \times 45.455}$$

$$Viscosity = \ 912.97\ gcm^{-1}sec^{-1}\ (\pm 0.7051)$$

The uncertainty of the radius of the ball is 0.01 cm. The uncertainty of the density of the liquid is 0.501 gmL^{-1}. The uncertainty of the density of the ball is 0.004 gmL^{-1}. The uncertainty of the velocity is 0.2 cm/sec. So, to calculate the uncertainty of the viscosity:

$$0.01^2 + 0.501 + 0.004 + 0.2 = 0.7051 gcm^{-1}sec^{-1}$$

Viscosity($\boldsymbol{\mu}$) ($gcm^{-1}sec^{-1}$) (±0.7051)												
Solution	**Repeat 1**	**Repeat 2**	**Repeat 3**	**Repeat 4**	**Repeat 5**	**Repeat 6**	**Repeat 7**	**Repeat 8**	**Repeat 9**	**Repeat 10**	**Mean** (±7.15)	**Standard Deviation** ($gcm^{-1}sec^{-1}$)
Mixture 1	679.91	618.10	618.10	618.10	679.91	618.10	679.91	679.91	618.10	679.91	649.01	32.57693
Mixture 2	494.48	556.29	494.48	556.29	494.48	494.48	556.29	494.48	432.67	556.29	513.03	41.71882
Mixture 3	494.48	556.29	494.48	556.29	494.48	556.29	556.29	556.29	494.48	494.48	525.39	32.57693
Mixture 4	618.10	556.29	618.10	556.29	618.10	618.10	556.29	556.29	556.29	618.10	587.20	32.57693
Mixture 5	556.29	556.29	556.29	679.91	556.29	679.91	679.91	556.29	556.29	556.29	593.38	59.7145
Mixture 6	618.10	679.91	618.10	618.10	618.10	618.10	556.29	679.91	618.10	679.91	630.47	39.09231

Table 5: Table to show the Viscosity (μ) for each repeat of each solution

The standard deviation can be found using the excel function. The deviation can be used because there are more than 5 repeats.

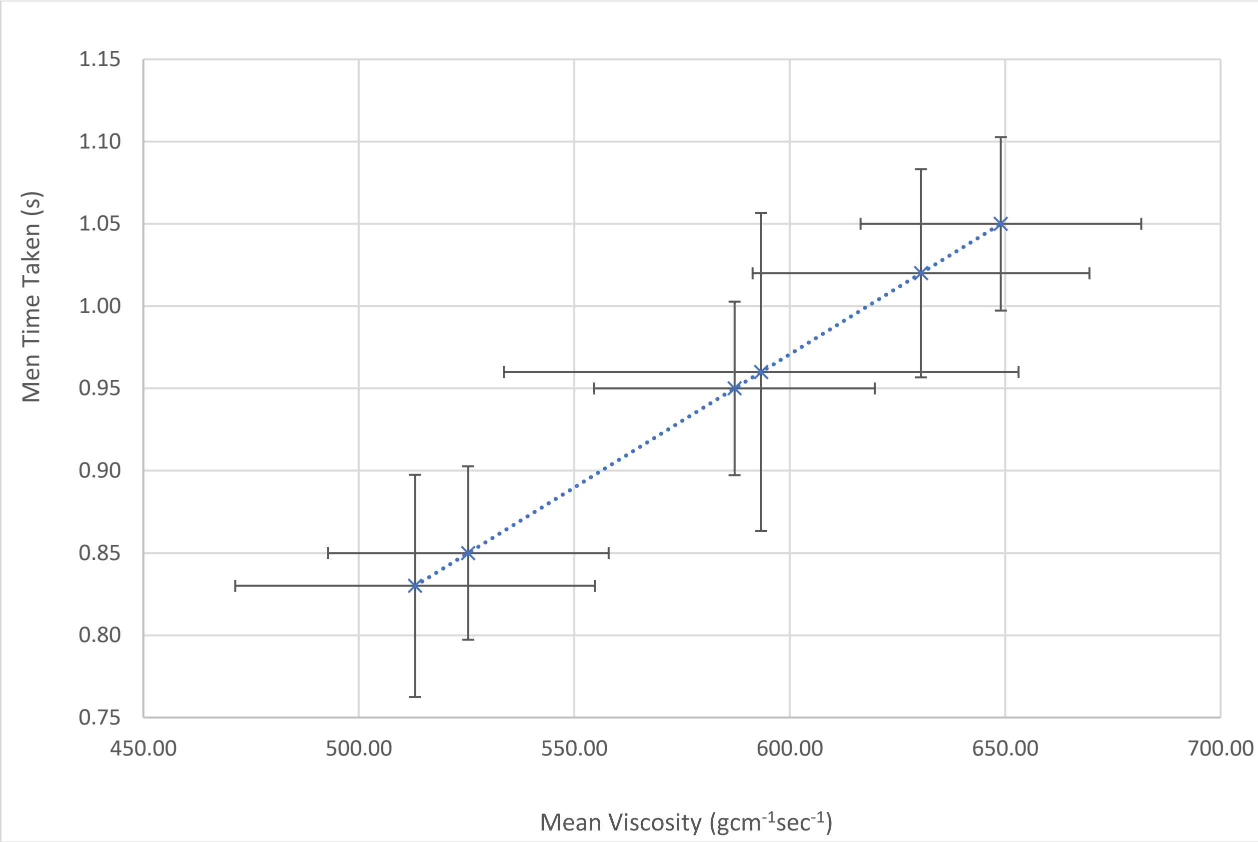


Figure 16: Line graph to show mean viscosity (gcm^{-1}sec^{-1}) against time (s) for each solution

This graph shows a direct correlation between the time taken for the ball to fall and the viscosity of the mixture. Hence, we can determine that as time taken for the ball fall increases, the viscosity of the mixture also increases. The x and y error bars show the standard deviation of the viscosity and the time respectively. A linear plot can be seen; thus, a direct correlation can be made with viscosity and time.

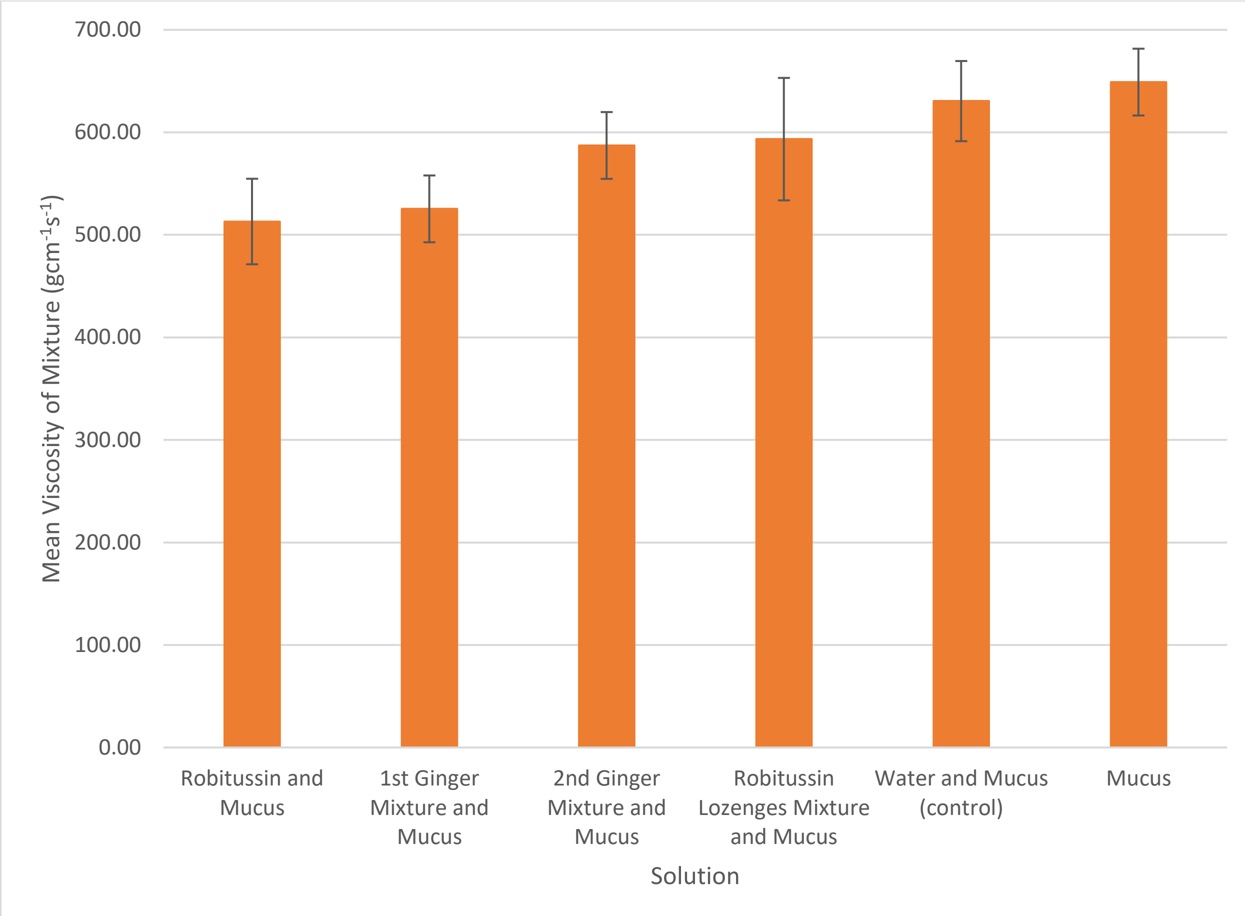


Figure 15: Bar Chart to show the mean viscosity (gcm^{-1}sec^{-1}) for each solution

It is visible that the error bars for the robitussin and mucus, 1st ginger mixture and mucus do not cross the error bars of the mucus. This suggests there was a clear difference made through adding these 2 solutions. The control experiment is almost identical to the mucus bar which further emphasises the difference the robitussin and 1st ginger mixture made.

9.0 Statistical Analysis

Excel by Microsoft was used to carry out the single factor ANOVA analysis, to see if there was significant variation between the viscosity in the different groups. The single factor ANOVA Test can be used to analyse the variance between 2 or more independent groups of data. There are 6 different independent groups (mixtures) present in this experiment, and ANOVA can help show the variance between these groups. The analysis can tell us whether the variation between the two or more groups is "significant".

Groups	*Count*	*Sum*	*Average*	*Variance*
Robitussin and Mucus	10	5130.261	513.0261	1740.46
1st Ginger Mixture and Mucus	10	5253.882	525.3882	1061.256
2nd Ginger Mixture and Mucus	10	5871.986	587.1986	1061.256
Robitussin Lozenges Mixture and Mucus	10	5933.796	593.3796	3565.821
Distilled water and Mucus (control)	10	6304.658	630.4658	1528.209
Mucus	10	6490.089	649.0089	1061.256

Table 6: Table made by Excel showing the variance of viscosity in each group

ANOVA Results			
Source of Variation	*F*	*P-value*	*F crit*
Between Groups	17.90847	1.97E-10	2.38607

Table 7: Table made by Excel showing the F and critical value for variance

- If the F value is greater than the critical F value, thus the null hypothesis can be rejected.
- The P-value determines whether the means are different. If the value is smaller than 0.05, then the means can be proven as varying and the null hypothesis can be rejected. *(One Way (Single Factor) ANOVA Example in Excel | QI Macros, 2019)*

10.0 Evaluation

10.1 Strength

When looking at the graphs, it is visible that the error bars of viscosity of Robitussin PS and the 1st ginger mixture do not overlap the control (water and mucus) or the mucus bars. This helps prove that these results were not a coincidence. This is further proven through the F value 17.91) in the ANOVA analysis being greater than the critical value of F (2.39) and the P value (1.97×10^{-10}) being smaller than 0.05.

Having ten repeats in this experiment helps improve the reliability of this experiment and also allows me to use the standard deviation as a means of comparison between the data.

Another observation that proves reliability for this experiment is shown in *figure 12*. The Robitussin syrup was below the mucus mixture, proving Robitussin PS was denser (had more atoms per cubic centimeter). However, when mixed, the Robitussin PS and mucus mixture actually lesser in viscosity, compared to the artificial mucus by an average of 20.95%. This proves that the cough remedy did have an expectorant effect on the artificial mucus. This is further proven through the negative control not having a significant effect on the artificial mucus (*figure 15).*

10.2 Weaknesses

Though the artificial mucus was very similar to actual mucus, it was not an exact representation of tracheal mucus. There were a few reasons for this: Mucin, a prominent ingredient in mucus, could not be purchased. This was because it was above the budget and wasn't worth it as the results were still significant without it.

Furthermore, using eggs instead of various different mucoproteins made the solutions slightly heterogenous. This could have varied the results to make one solution of mucus more viscous than another. Tap water is also not homogenous, which could have also varied the viscosity of the mucus.

The uncertainty of a stopwatch is 0.01s, however by slowing down the video, the uncertainty of the stopwatch to 0.05s as the interval increased to 0.1s per frame. The dosage of the active ingredient could have varied for each mixture, as each substance had a different active ingredient and concentration. Also, the amount of each constituent in not known for a certain mass of ginger. Because of time and law constraints, not all ingredients could be compared to a suppressant experimentally. Only the active ingredients were compared. Mucus thinning does not have to relate to the ease of cough. After a certain amount of thinning mucus, it could be harmful to the body.

11.0 Conclusion

Using this experiment to answer the research question, it can be known that Robitussin PS has the best expectorant properties out of the medicines tested on. The F value in the ANOVA test is greater than the critical F value *(table 7)*. This data conforms to the hypothesis stated in this experiment.

Robitussin PS and the 1st ginger mixture were known to be a significantly lower viscosity compared to artificial mucus and the negative control. This also leads to the rejection of the null hypothesis.

Part of the hypothesis was proven incorrect, as the boiling water and ginger did not have as significant as a decrease compared to room temperature and water. This was surprising as warm water and ginger is commonly used in households to relief a cough. A reason for this may have been because the active ingredient, *Guaiacol*, has melting point been around 32 ^{o}C *(PubChem, 2019),* which means its expectorant properties would have decreased once boiling water was used. The error bars did overlap, so this could be proven wrong if more repeats were taken.

Ginger is not as good of an expectorant as Robitussin PS, as stated in the hypothesis and proven by this experiment. This may be because Robitussin PS is more concentrated with the expectorant compared to ginger. An improvement could be the nano formulation of a constituent of *Zingerone*, *Gingerenone A* and *Gingerol*. Research papers have been published with the nano formulation of *Gingerol (Wei et al., 2018)*, which would increase the amount of expectorant that can be supplied per ml of ginger mixture. This could help truly decided whether ginger could be a better solution for cough compared to other cough medicines. As shown in the structural comparisons, none of the ingredients used in this test had suppressant properties in their active ingredients. Adding an antitussive could be a disadvantage as it could be addictive.

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13.0 Appendix A: Cough Remedies and a Comparison of Antitussive Properties

13.0a Robitussin DM

Robitussin DM is a drug administered orally (as a syrup), which contains *Dextromethorphan* HBr. This is much different to an expectorant, as the constituents of a suppressant are assimilated to the brain and help increase the threshold of irritants in the throat or airway. This helps decrease the amount of coughing done by a person as their throat can hold more irritants without the need to get rid of them. However, *Dextromethorphan Hydrogen Bromide (HBr)* is only available with a prescription in Singapore, as it can be used as an anesthetic with higher doses and it linked to drugs such as ketamine for its opioid-like properties. Hence, it is not used in this experiment. This is the structure of *Dextromethorphan HBr*:

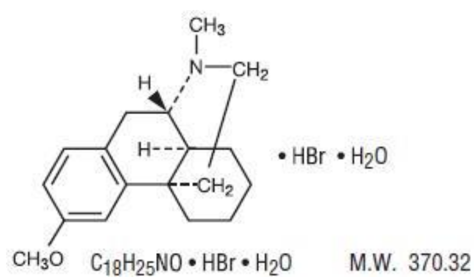

Figure 3: Figure showing the structure of Dextromethorphan HBr (Drugs.com, 2019)

This structure will be compared to the structure of other active ingredients to find any similarities and differences. Through this, assumptions on the effectiveness of those structure as antitussives can be made.

13.0b Robitussin PS

Guaifenesin AC is an active ingredient in many cough related drugs, including the one being used in this experiment (Robitussin PS syrup). This is the structure of *Guaifenesin*:

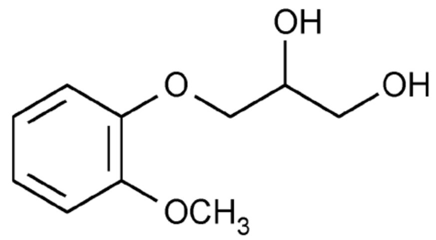

Figure 1: Figure showing the structure of Guaifenesin AC (RxList, 2019a)

The other active ingredient in Robitussin PS is *Pseudoephedrine Hydrochloride*, which has the structure:

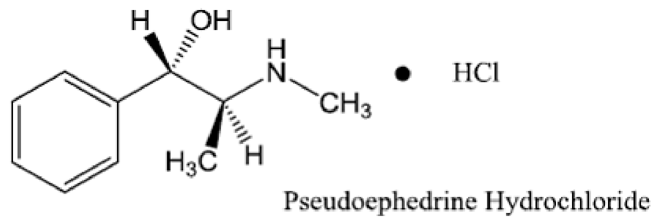

Figure 2: Figure showing the structure of Pseudoephedrine Hydrochloride (Rezira (RxList, 2019b)

When comparing Robitussin PS to *Dextromethorphan HBr*, both the active ingredients have to be considered. In *Figure 1*, it is possible to see that *Guaifenesin* includes the same benzene ring with the methoxy group. This makes *Guaifenesin* the expectorant it is. There are no more direct similarities that can be found between the 2 structures.

With the other active ingredient, *Pseudoephedrine Hydrochloride*, there are no similarities except for the benzene ring. This would make sense as Robitussin PS is made to have no suppressants in it, so it can be store-bought, compared to Robitussin Dm, which contains *Dextromethorphan HBr*. This is an opioid, thus Singapore does not allow the selling of this product without approval.

13.0c Robitussin Lozenges

Robitussin lozenges contain the active ingredient, *Amylmetacresol* and *2,4 dichlorobenzyl alcohol* (both antiseptics that help kill germs and bacteria). These lozenges help with sore throat and acute cough, by killing any pathogenic molecules present, mostly in the throat.

The structure of *Amylmetacresol* is:

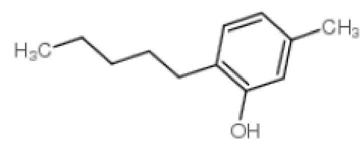

Figure 4: Figure showing the structure of Amylmetacresol (ChemSrc, 2019)

The structure of *2,4 Dichlorobenzyl Alcohol* is:

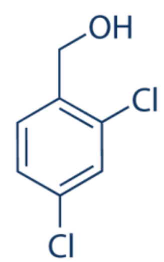

Figure 5: Figure showing the structure of 2,4 dichlorobenzyl alcohol (Selleckchem, 2019)

When comparing *Amylmetacresol* (1 of 2 active ingredients in Robitussin Lozenges) in *Figure 4*, one can see *Guaiacol* present, however this is not the same as Anisole, which was the same problem with ginger. Except for this there are no similarities found. Using *Figure 5* to compare the 2nd active ingredient, *2,4 Dichlorobenzyl Alcohol*, the only similarity that can be seen is the phenol group present but there are 2 carbon-chlorine bonds present as well.

13.0d Ginger

Using the NIH's National Center for Biotechnology Information, it is possible to find the constituents of store-bought ginger:

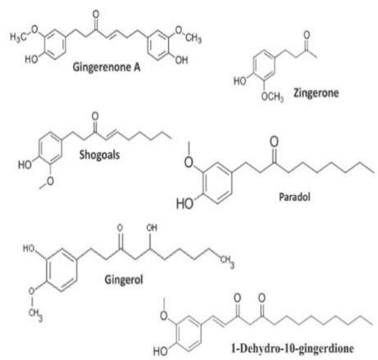

Figure 6: Figure showing the structure of the constituents of ginger (Townsend et al., 2013)

It is possible to see, from *Figure 6*, that some of ginger's constituents include the same hexagonal ring with 3 double bonds (a benzene ring) and an O-CH_3 (methoxy group) bond attached to it (with an extra OH bond), which similar to *Dextromethorphan*'s structure too (as seen in *Figure 2*). This methoxybenzene ring is called Anisole. However, when there is an extra OH bond (like there is with *Gingerenone A, Zingerone and Gingerol*), this is known as 1-methoxy phenol, or *Guaiacol*. However, there are no more similarities in structure.

It is made obviously that there are very little structural similarities between the active ingredients of the cough remedies being used in this experiment and *Dextromethorphan HBr*, the antitussive active ingredient of Robitussin DM.

13.1 Appendix B: Equipment List

Apparatus	Quantity and Uncertainty/Reason	Risks	Way(s) to reduce risks	What to do if the equipment breaks/doesn't work
Measuring cylinder	**x2 100 mL (±0.1)** **x1 50 mL (±0.5)** (To measure mixtures made and weigh them to find density) **x6 25 mL (±0.25)** (To measure tap water) **x3 10 mL (±0.05)** (To measure enzymes)	May fall and break.	Stand up when using the equipment.	Clean with broom and dustpan (carefully).
Metal ball and magnet	**x1** (To drop through the solution and pick back up)	May drop on foot.	Make sure to be standing up when dropping the ball.	Clean with broom and dustpan (carefully). More likely to lose the ball than break it. Replace if lost. (make sure mass is similar)
Beaker	**x1 250 ml** **x2 150 ml** **x4 50 ml** (To store the mixtures made)	May fall and break. Made of glass, could cut through skin.	Stand up when using the equipment.	Clean with broom and dustpan (carefully).

pH probe	**x1** (To measure the pH of the mucus solution, which needs to be around 7)	May malfunction or break if it falls.	Hold the probe the entire time during use.	Test to see if still working, if not tell teacher.
Mass balance	**x1** (To measure the mass of different constituents)	May malfunction or break if it falls.	Make sure it is not on the edge of the table and it carefully transported if moving	Test to see if it is working, if not consult teacher.
50 mL Burette	**x1** (Represents 66.5 cm (0.1) distance to drop ball from)	May fall and break. Made of glass, could hurt.	Keep steady to clamp stand and make sure it is at eye level so there is lesser risk.	Clean with broom and dustpan (carefully).
Retort Stand and Clamp	**x2** (To hold burette and device to video the ball dropping)	May drop on foot.	Keep away from the edge of the table so it can't fall onto the ground. Hold steady whenever one is setting it up.	Try and fix parts together. Consult teacher if not possible.
Stopwatch	**x1** (As a representation to be able to time the ball in the slow-motion video)	If the stopwatch breaks, the timing of another stopwatch may be different.	Make sure the stopwatch stays attached to the clamp stand and the clam stand is not near the edge of the table.	Consult a teacher to replace the equipment.
iPhone X	**x1** (To record the burette as the ball drops)	Could fall from the clamp stand. Would be	Make sure the phone stays attached to the clamp stand and the	Consult a teacher to use a different equipment.

		expensive to replace Have to keep the phone at the same place, so avoid parallax error.	clam stand is not near the edge of the table.	
Kapwing Video Editor	Online software to slow down the video recording	File could get corrupted while slowing the video down.	Follow procedure as stated on the kapwing editor.	Consult a teacher on which editor to use instead.

Table 1: Table showing the apparatus used and the safety precautions taken for the necessary hazard

13.2 Appendix C: Calculations

13.2a Calculating the density (ρ) (in gcm^{-3}) of each solution

$$Density(\rho)\ (gcm^{-3}) = \frac{Mass\ (g)}{Volume\ (cm^3)}$$

13.2b Calculating the density (ρ) (in gcm^{-3}) of the ball

Volume of a sphere:

$$Volume\ (cm^3) = \frac{4}{3}\pi r^3$$

The diameter of the ball is 5 mm (±0.01), so the radius is 0.25 cm (±0.001)

Volume of the metal ball:

$$Volume\ (cm^3) = \frac{4}{3}\pi \times 0.25^3$$

$$Volume = 0.0655\ cm^3\ (\pm 0.003)$$

Mass of the ball: 0.265 g (±0.001)

Density(ρ) of Ball: 4048.9 gcm^{-3} (±0.004)

The uncertainty is found by summing the uncertainty of the mass (0.001) and the volume (3 multiplied by the radius: 0.003).

13.1c Calculating the velocity (v) of the ball

$$Velocity\ (v)(cmsec^{-1}) = \frac{Distance\ (66.5\ cm)}{time\ taken\ (s)}$$

13.2f Calculating the viscosity of each repeat for each solution

Using Stoke's Law equation rearranged for liquid viscosity($\boldsymbol{\mu}$):

$Viscosity\ (\mu)(gcm^{-1}sec^{-1})$

$$\frac{2 \times g\ (981\ cmsec^{-2}) \times r\ of\ ball\ (cm)^2(\ \rho\ (disperse\ phase)\ (gcm^{-1}) - \rho\ (continuous\ phase)(gcm^{-1})}{9\ \times v\ (cmsec^{-1})}$$