Analysis of Milk

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Abstract

Milk is a very important part of daily diet all around the world. It is also used as an ant-acid and is a good source of calcium for humans. This experiment deals with measuring the buffering capacity of milk, observing the process of digestion of milk by Trypsin and determining the calcium content of a given sample of milk.

[Note: This experiment is in 3 parts and teachers can feel free to take the parts individually to students as per their need. The three parts are: pH measurement, milk digestion and measurement of calcium content]

This experiment was the longest of all the experiments designed for the 10th International Junior Science Olympiad that was held at Pune. It is in three parts and teachers can split it into 3 different experiments as per their requirements. The “marks” listed are only a guide and teachers are free to assign marks as per their requirement. This experiment was designed keeping in mind the integrated nature of science and, at the same time, connecting it to the Indian context. Milk has been one of the most important components of diet in India and, hence, carrying out experiments on milk that invoke biology, chemistry and physics concepts are of interest and importance. The physics aspect of the experiment, which was to measure the density of milk, was dropped as the experiment was getting too lengthy. However the density measurement of milk will be dealt with separately in a future publication. The biology and chemistry aspects of the experiment were, however, very intricately mixed and, indeed, were supported by several principles of physics. It was also attempted to use equipments where students can see the principles of science at work while doing the experiment. The intricate procedures were designed to test the ability of students to follow the procedures correctly to achieve the desired results.

India is one of the largest milk producing countries in the world. A large part of the credit for this goes to the world’s biggest agricultural development programme, Operation Flood, initiated and sustained by **Dr. Verghese Kurien**, known as the “Father of the White Revolution” for his billion-litre idea.

The buffering capacity of milk

Milk is a source of many nutrients. It consists of 87% water and 13% solids suspended or dissolved in water, in the form of proteins (3.5%), carbohydrates (4.7%), fats (4.0%) and vitamins/minerals (0.8%). The major milk sugar is lactose, which is water soluble. Milk fat is in the form of globules emulsified in water. The most abundant protein in milk is casein, which exists as a suspension of particles called casein micelles. Each micelle consists of thousands of casein molecules; the micelles are, in turn, bound together by Ca^{2+}. The casein micelles and fat globules give milk its white colour and deflect light rays passing through it. Milk is slightly acidic with a pH between 6.4-6.8. Curdling of milk occurs when the pH of milk is reduced to 5.0. At this pH, the milk casein molecules clump together and precipitate. Milk is known to have a good buffering capacity.

This experiment is designed for the students (and teachers) to measure this capacity of milk to resist change in pH. In this experiment, comparison is made between water and milk in terms of change in pH upon addition of acid (or base). The quantity of acid (or base) required to change the pH of milk (or water) to a desired level, is studied.

It is **essential** in this experiment that we use **toned milk**[*] since the globules of fat will

[*] ***Toned milk*** *is a method, developed in India, of treating buffalo* ***milk*** *by adding skim* ***milk****, powdered skim* ***milk*** *and water to buffalo* ***milk****. This process decreases the fat content, increases the quantity of available* ***milk****, and 'tones up' the non-fat solids level to the original amount. [Wikipedia]*

have their own reactions and can occult the processes we are planning to study. In case of presence of fatty acids, the experiment will have to be redesigned with more sensitivity.

This experiment has a feature where the laboratory equipment is labeled appropriately for the students to identify the ingredients and equipment easily. Using proper equipment for measurements is also a training aspect for students and teachers of science. Measuring pH with pH meters will result in higher accuracy of the results. But at school level using pH paper also gives desired results with relative ease and no compromise in the subjective conclusion for the students.

To ensure that all the students get same source of milk, and is free of any added contaminations (!), it was planned to use milk powder to be mixed with water as and when required to produce milk for the experiment. The unused milk powder can be stored in a refrigerator for reasonably long time, for future experiments, without fear of putrification.

You are supplied with the following:

	Labeled as ….	**Quantity Supplied**
Milk	**Milk**	100 ml in red cap plastic jar
3% (v/v) acetic acid solution	**AA**	10 ml in sample container AA
3% (w/v) sodium carbonate solution	**SC**	10 ml in sample container SC
Water bottle	**Water**	1000 ml in bottle
100 ml glass beakers	**W, Exp**	2
20ml graduated syringe	**A**	1
1 ml graduated syringes	**B, C**	2
pH papers; range 2 to 10.5		2 booklets
Wash bottle		1
Glass rod		1
Tissue roll and Waste bucket		1 each

Procedure

1. Pour water from the water bottle into the beaker **W** until it is roughly full.
2. Transfer 40 ml of water into the beaker **Exp,** using syringe **A**.
3. Measure the pH of the water in beaker **Exp.** For this, dip the given pH paper strip in the water in the beaker for a few seconds. Take out the dipped pH paper and observe the colour change; match the colour with the pH range provided on the booklet. Write the pH in the answer sheet. [Q1 0.25 marks]
   [Here the teachers have to observe the students carefully. While removing the pH paper from the booklet they tend to touch the bottom portion with hand and use the same portion to dip in the given liquid to test pH. This can result in contamination.

Even if the contamination is less, nevertheless it inculcates a culture of doing careful experiments. Also dipping the pH paper in water or any solution for more than 2 seconds results in pH paper reacting with the solution and possibly contaminating it in some way.]

4. Measure the pH of sodium carbonate solution supplied in the sample container **SC**. Write the pH in the box in the answer sheet. [Q2 0.25 marks]
5. Add 0.1 ml of sodium carbonate solution to the water in beaker **Exp** using syringe **B**. Stir well with the glass rod and measure its pH with a pH paper. Write the new pH value observation Table B.1 in the answer sheet.
6. Continue adding 0.1 ml of sodium carbonate solution and write the pH values in Table B.1 in the answer sheet, till the pH of the solution reaches 10. Also, write the total volume of sodium carbonate solution added. [Q3 1.0 mark]

   [It is quite impractical to add sodium carbonate till the pH of water reaches that of sodium carbonate. So a reasonable pH value of 10 was chosen so that the results can be achieved in reasonable time.]
7. Now wash the beaker Exp and glass rod so that no traces of the previous solution remain. Wipe it with tissue paper.
8. Add 40 ml of water in to the washed beaker Exp using syringe A.
9. Measure the pH of acetic acid in sample container AA. Write the pH in the box in the answer sheet. [Q4 0.25 mark]
10. Add 0.1ml of given acetic acid solution to the water in beaker Exp, using syringe C. Stir well with the glass rod and measure the pH with a pH paper. Record the pH value in the Table B.1 in the answer sheet.
11. Continue adding 0.1ml of acetic acid solution and write the pH values in Table B.1 in the answer sheet, till the pH of the solution reaches 4. Also write the total volume of acetic acid solution added. [Q5 1.0 mark]
12. Now wash the beaker Exp and glass rod so that no traces of the previous solution remain. Wipe it with tissue paper.
13. Use syringe A to add 40 ml of milk to the washed beaker Exp.
14. Measure the pH of the milk using the pH paper. Write the pH in the box in the answer sheet. [Q6 0.25 marks]
15. Using syringe B, add 0.5 ml of sodium carbonate solution to the milk in beaker Exp. Stir well with the glass rod and measure the pH. Write the pH value in Table B.2 in the answer sheet.
16. Keep adding 0.5 ml of sodium carbonate solution till the pH value of the milk sample reaches 10.
17. Write the pH value for each addition in observation Table B.2 in the answer sheet. Also write the total volume of sodium carbonate solution added. [Q7. 1.0 mark]
18. Now wash the beaker Exp and glass rod so that no traces of the previous solution remain. Wipe it with tissue paper.
19. Use syringe A to again add 40 ml of milk in to the washed beaker Exp.
20. Using syringe C, add 0.5 ml of acetic acid solution to the milk in beaker Exp. Stir well with the glass rod and measure the pH. Keep adding 0.5 ml of acetic acid solution till the pH value of the milk sample reaches 4.

21. Write the pH value for each addition in observation Table B.2 in the answer sheet. Also write the total volume of acetic acid solution added. [Q8. 1.0 mark]
22. Wash the beaker Exp and glass rod, dry it with tissue, and keep it ready for the next task.

Questions

From your observations in Tables B.1 and B.2, write on the answer sheet whether the following two statements are true (T) or false (F). [Q9 1.0 mark]

a) You require more acetic acid solution to lower the pH of milk to 4 than to lower the pH of water to 4.
b) You require less sodium carbonate solution to raise the pH of milk to 10 than to raise the pH of water to 10.

As compared to water, milk resists change in pH of the resulting solution when acetic acid is added. This is because components of milk:

a) lead to increase in concentration of the OH^- ions in the resulting solution
b) prevent increase in concentration of the free H^+ ions in the resulting solution
c) lead to decrease in concentration of CH_3COO^- ions in the resulting solution

Write the correct option in the appropriate box in the answer sheet. [Q10 1.0 mark]

[**Caution for teachers:** In this experiment it is very essential to see that whenever asked, student wash the beakers and dry it with utmost care. Any remnants in the beaker will contaminate the next part. To avoid this one can use different beaker for every part but it only increases the number of equipment a student has to handle

It is also essential for teachers to observe that students avoid touching that portion of pH paper, which is going to be dipped in the sample for testing. Contaminants from the surface of skin can lead to erroneous results. Another way to avoid this contamination is wearing of latex/cotton hand gloves

It is also essential for teachers to observe that students do not keep the pH paper dipped in solution for a long time to soak the pH paper completely.**]**

Enzymatic digestion of milk protein

To measure the change in opacity of milk due to digestion of milk proteins with trypsin (a protease)

Addition of trypsin to milk breaks down casein. This causes the milk to become translucent. The rate of reaction can be measured by determining the time it takes for the milk to turn translucent. You will use a photodiode in your measurements. A photodiode is a device that converts light into electrical current which you will measure using a digital multimeter. You will also use a light emitting diode (LED) as a light source.

You are supplied with the following:

	Labeled as...	**Quantity Supplied**
Power supply; 500 mA, 3 V		1
Acrylic set-up with photodiode (see an image below)		1
White LED		1
Digital multimeter		1
Test tube	**ED**	1
Milk		As supplied for Task B1
Trypsin	**TE**	5 ml in a test tube
Water		As supplied for Task B1
Graduated syringe(1ml)	**TE**	1
Graduated syringe (12 ml)	**W**	1
Stop watch		1
Dropper		1
Sticky paper		

Procedure

1. Mount the White LED in the space provided on the fixed part of the acrylic stand, as shown in the photograph above. You may have to use sticky paper provided to you to ensure that the LED is mounted tightly.
   [The acrylic stand has been defined in greater details in IAPT bulletin of October 2014]
2. Connect the White LED to the Power supply such that shorter leg of the LED connects to black wire. Then switch the power supply on. The LED should glow brightly.
3. Set the multimeter in the current mode and 2 mA current range.
   [This is not a rigid requirement but just to get the students started].
4. Connect the photodiode mounted on the movable part of the acrylic stand to the multimeter.
5. Add 10 ml of water to test tube ED using syringe W*;* use tissue paper to wipe the outer surface of ED so that it is completely dry. Then place the test tube in the space provided for it on the acrylic stand.
6. Ensure that the light from the LED passes through the water in the test tube and falls on the photodiode. Orient the test tube such that the light is not blocked by the label.
7. Adjust the positions of the photodiode and test tube by carefully sliding either the mounted photodiode or the test tube holder such that the current reading on the multimeter maximizes. Record the maximum current I_W in the answer sheet. If required you are free to change the range of measurement of current, set on the multimeter.

[Q11. 0.5 mark]

Note that for subsequent readings these positions of the photodiode and test tube holder must remain the same.

[Here the teachers should test the carefulness and skill of students in trying to place the components in right place]

8. Remove the test tube from the acrylic stand and pour out the water.
9. Add 5 ml of water in test tube ED and then add 5 ml of milk to it with the help of syringe W. Mix well by gently tapping the test tube. Wipe the outside of the test tube with tissue paper to ensure that it is dry. Carefully place the test tube in the space provided on the acrylic stand and record the current I_0 in the answer sheet. [Q12 0.5 mark]
10. Keep the stopwatch ready to start.
11. Use syringe TE to add 1 ml of trypsin to this milk sample in the test tube. Mix thoroughly using the plastic dropper. Ensure that test tube holder stand is at its original place (where previous readings were taken). If the trypsin is added with force, by applying excessive pressure on syringe, mixing by dropper is not necessary.
12. Immediately start the stopwatch.
13. Read the current on the multimeter at 15 seconds intervals and record the values in Table B.3 in the answer sheet.
14. Continue recording the values of current up to 7 minutes. [Q13. 2.0 marks]
15. Discard the solution and wash the test tube.

Graph plotting

Plot a graph of current versus time in the grid provided in the answer sheet.

[Q14. 3.5 marks]

Questions

Mark a point K on the graph where the casein concentration is maximum, a point L where the casein concentration is minimum, and a point M where the casein concentration is half-way between maximum and minimum values. [Q15. 1.0 mark]

If the increase in current is proportional to the amount of digested casein and maximum current represents complete digestion of casein, deduce from the graph the time taken for digestion of 50% casein. [Q16. 1.0 mark]

[Caution for teachers Here teachers have to ensure that the current ranges are set appropriately and the connections are in current mode of multi meter (and not in voltage mode). Teachers have to explain to students the difference of these two modes of connection on a multimeter. Wrong connection will give completely false set of numbers which will make no sense. Some multimeters have 2A and 10A optons. At school level 10A option is never to be used.

Teachers should also ensure that students have kept the test tube, the light source and the detector at appropriate place

In case the stop watch is switched ON a few seconds after trypsin is poured into the mild, it will not change the nature of the graph. However, stirring of trypsin in the milk, using a dropper, is very essential for the reaction to occur as desired.

If a student does not stir the contents, it is possible that trypsin will digest the milk in the upper portion of the test tube and then slowly diffusing towards the lower sections of the test tube, giving a completely different result on the I vs t graph and confusing the student.]

B3 Estimation of calcium content in milk

It gives a thrill to a student of class X to do an experiment which whose result closely matches the numbers written on the containers of the milk (or its powder in this case).

Calcium content in milk can be estimated by a special form of titration using a reagent called **Na_2EDTA**. Na_2EDTA reacts with metal ions in 1:1 proportion irrespective of the charge on the metal ion. Indicators used in such titrations are called metal-ion indicators. The indicator used in the present experiment is Eriochrome black T **(EBT)**.

You are supplied with the following:

	Labeled as ...	**Quantity Supplied**
Trypsin-treated milk	**CM**	100 ml in a volumetric flask
Water		As supplied in task B1
100 ml glass beaker	**HM**	1
10 ml graduated syringe	**CM**	1
100 ml conical flask	**HM**	1
Buffer solution pH 10	**BF**	Three 5 ml test tubes with screw caps
Dropper		1
Eriochrome Black T indicator	**EBT**	Dropping bottle
Burette 25 ml (on a stand)		1
Na_2EDTA solution (0.0027 M)	**EDTA**	80 ml in plastic bottle
Funnel		1

Procedure:

1. Add the Na_2EDTA solution to the burette using the funnel.
2. Write the initial burette reading in Table B.4 in the answer sheet
3. Dilute the given trypsin-treated milk in the volumetric flask CM with water up to the mark. Insert the stopper and shake the solution well to homogenize it.
4. Now pour out the homogenized solution into beaker HM.
5. Add 10 ml of homogenized solution, using syringe CM, to the conical flask HM.
6. Add 10 ml of water to it, using syringe W.
7. Now add all the supplied buffer amount from *one* of the test tubes BF.

8. Add 5 drops of EBT indicator from the dropping bottle. The colour of the solution will change to red (pinkish red).
9. Titrate this solution in the conical flask HM with Na_2EDTA from the burette. Continue till the colour of the solution changes initially to purple and then to the first appearance of blue (which is the end point).
10. Write the final burette reading in Table B.4 in the answer sheet
11. Repeat the titrations twice.
12. Enter your readings in the observation Table B.4 in the answer sheet.
13. Calculate the volume of the solution needed for titration I, II and III. Write the values in Table B.4 in the answer sheet.
14. Calculate the average volume. [Q17. 3.5 marks]

Question:

Deduce the amount in milligrams of Ca^{2+} per 10 ml of the diluted solution (the atomic weight of Ca is 40). **[Q18.1.0 mark]**

B1 The buffering capacity of milk

B.Q1.A pH of water = ☐ **[0.25 Mark]**

B.Q1.B pH of sodium carbonate = ☐ **[0.25 Mark]**

B.Q1.C pH of acetic acid = ☐ **[0.25 Mark]**

B.Q1.D pH of milk = ☐ **[0.25 Mark]**

B.Q2 Observation Table B.1 **[2.0 Marks]**

	Stepwise addition to 40 ml water			
	Sodium carbonate solution		Acetic acid solution	
	Stepwise volume added in ml	**pH** value	Stepwise volume added in ml	**pH** value
1	0		0	
2	0.1		0.1	
3	0.1		0.1	
4	0.1		0.1	

5	0.1		0.1	
6	0.1		0.1	
7	0.1		0.1	
Total	……	Volume of Na_2CO_3 solution added to reach pH 10.0	……	Volume of CH_3COOH solution added to reach pH 4.0

B.Q3 Observation Table B.2 **[2.0 Marks]**

	Stepwise addition to 40 ml Milk			
	Sodium carbonate solution		Acetic acid solution	
	Stepwise volume added in ml	**pH** value	Stepwise volume added in ml	**pH** value
1	0		0	
2	0.5		0.5	
3	0.5		0.5	
4	0.5		0.5	
5	0.5		0.5	
6	0.5		0.5	
7	0.5		0.5	
Total	……	Volume of Na_2CO_3 solution added to reach pH 10.0	……	Volume of CH_3COOH solution added to reach pH 4.0

Questions:

B.Q4 **[1.0 Mark]**

Comparing the observations in Table B.1 and B.2 which of the following statements describe the role played by milk?

a) You require more acetic acid solution to lower the pH of milk to 4 than to lower the pH of water to 4.

True (T) ☐ False (F) ☐

b) Less sodium carbonate solution is required to raise the pH of milk to 10 than to raise the ph of water to 10

True (T) ☐ False (F) ☐

B.Q5 **[1.0 Mark]**

As compared to water, milk resists change in pH of the resulting solution when acetic acid is added. This is because components of milk:

d) lead to increase in concentration of the OH^- ions in the resulting solution
e) prevent increase in concentration of the free H^+ ions in the resulting solution
f) lead to decrease in concentration of CH_3COO^- ions in the resulting solution

Write the correct option in the appropriate box [ ]

Enzymatic digestion of Milk protein

B.Q6.A I_w = [ ] **[0.5 Mark]**

B.Q6.B I_O = [ ] **[0.5 Mark]**

B.Q7 **Observation Table B.3** **[2.0 Marks]**

	Time (in s)	**Current (in mA)**		**Time (in s)**	**Current (in mA)**
1.			16.		
2.			17.		
3.			18.		
4.			19.		
5.			20.		
6.			21.		
7.			22.		
8.			23.		
9.			24.		
10.			25.		
11.			26.		
12.			27.		
13.			28.		
14.			29.		
15.			30.		

B.Q8 **Graph plotting:** **[3.5 Marks]**

B.Q9 **[1.0 Mark]**

Mark a point K on the graph paper where the casein concentration is maximum, a point L where casein concentration is minimum and a point M where the casein concentration is half-way between the maximum and minimum values.

B.Q10 **[1.0 Mark]**

If the increase in current is proportional to the amount of digested casein and maximum current represents complete digestion of casein, deduce from the graph the time taken for digestion of 50% casein.

B3 Estimation of calcium content in milk

B.Q11 Observation Table B.4 **[3.5 Marks]**

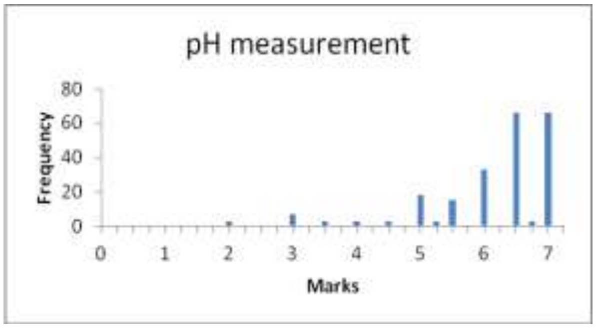
Sr. No.		Titration 1	Titration 2	Titration 3
1	Initial burette reading ml			
2	Final burette reading ml			
3	Difference in burette reading ml			

Average burette reading: (A)…………………………………ml of 0.001 M Na_2EDTA

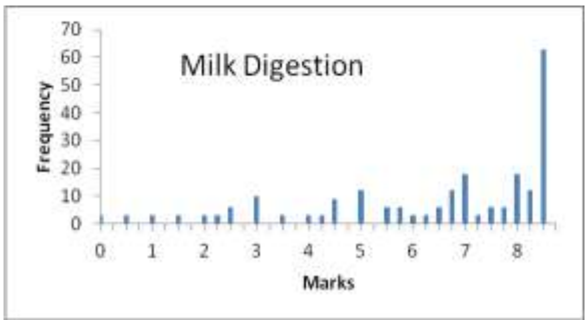
B.Q12 **[1.0 Mark]**

Deduce the amount in milligrams of Ca^{2+} per 10 ml of the diluted solution (the atomic weight of Ca is 40).

Results:



In December 226 students who participated in the 10th IJSO held at Pune. The results of the three parts of experiment are listed below. In the first part pH measurement 135 students scored more than (or equal to) 6.5 out of 7.0. The distribution of marks obtained can be seen in figure below.



In the second experiment where digestion of milk was carried out, 93 out of 226 students received more than 8 (or equal) marks out of 8.5 . The distribution of marks can be seen in the figure attached.

It can be seen that remaining 133 students did not get it fully correct. This is usually the case where the solutions are not prepared precisely as per the procedure. Several students also lost marks due to the improper graph procedures, i.e. representation of the results obtained, which is an integral part of experiments.

In the third part, only 69 students received 4 (or equal) marks out of 4.5. Ideally the milk digested in part 2 could have been used to determine the calcium content in part 3. However, an error in part 2 can have cascading effect in part 3. So in order to avoid double penalty to students, of this age group, they were provided with standard digested milk. However, in spite of that the distribution of marks was evenly spread out over all range of marks. About 10% students did not attempt this question. which was not the case in part I and II.