

Task: B Milk (20 marks for this task)

Examination Rules:

1. You are not allowed to bring any tools **except** any personal medicine or any personal medical equipment.
2. You must sit at your designated table.
3. Before the examination starts, you must check the stationery and any tools (pen, ruler, calculator) provided by the organizers.
4. You must check the question paper and answer sheet. Raise your hand, if you find any missing sheets. You may start only when given the signal by the organizers.
5. During the examination, you are not allowed to leave the examination room except in an emergency and for that the examination supervisor/volunteer/invigilator will accompany you.
6. You are not to disturb any other competitor or disrupt the examination. In case any assistance is needed, you may raise your hand and the nearest supervisor will come to help.
7. You may not question or discuss the examination problems with anyone other than your team members. You must stay at your table until the time allocated for the examination is over, even if you have finished the examination or you do not want to continue working.
8. A signal will indicate the end of the allotted time for the examination. You are not allowed to write anything on the answer sheet after the allocated time is over. You must leave the room quietly after all the answer sheets have been collected.

Read the following instructions carefully:

1. While you are in the examination hall, you should wear safety spectacles at all times. While doing your experimental task, always wear your lab coat, safety goggles, and hand gloves.
2. Handle each and every apparatus and chemicals with care.
3. Do not try to taste or smell any chemical substance.
4. Chemicals are very safe if handled and disposed of properly.
5. Ensure that you keep the answer sheet and question paper away from liquids.
6. Place all waste papers and used material in the waste basket provided.
7. Immediately report all accidents, injuries, however minor they may be, to the invigilator/supervisor/volunteer present.
8. Eating of any kind of food is strictly prohibited during the experimental task.
9. You are expected to work safely, to behave socially, and to keep the equipment and work environment clean. When carrying out discussions with your teammates, keep your voice low.
10. Do not leave the examination hall until you have permission to do so. Ask an invigilator/supervisor/volunteer if you need to use the bathroom.
11. You may start working only when the start signal is given.
12. You have 3 hours to complete the experimental tasks and to record your results on the answer sheets. You must stop your work immediately after the stop command is given.
13. Be sure that your team has a complete set of the question paper (3 copies) and 2 types of answer sheets (1 white copy for rough work and 1 yellow copy for final answers).
ONLY YELLOW ANSWER SHEETS WILL BE EVALUATED.
14. Use only the pen and calculator provided.
15. ID code must be written on every page of the final (yellow) answer sheets. Each team member must sign on the front page of the final (yellow) answer sheets.

Experimental Tasks



Time : 3 hrs
Marks :40

16. All results must be written in the designated boxes on the **yellow answer sheets**. **Data written elsewhere will not be graded.**
17. After completing the task, put all the equipment back to its original place. Make sure you clean your work place.
18. After the stop command is given, put all papers inside the envelope kept on the desk. Wait for the volunteer to check and collect it.

Task B: In this set of experiments we will investigate,

- B1 The buffering capacity of milk.
- B2 Enzymatic digestion of milk proteins.
- B3 Estimating the calcium content of milk.

B1 The buffering capacity of milk

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.



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.

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

- Pour water from the water bottle into the beaker **W** until it is roughly full.
- Transfer 40 ml of water into the beaker **Exp**, using syringe **A**.
- 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 leaflet. Write the pH in the **box in the yellow answer sheet**.
[B.Q1.A: 0.25 marks]
- Measure the pH of sodium carbonate solution supplied in the sample container **SC**. Write the pH in the **box in the yellow answer sheet**.
[B.Q1.B: 0.25 marks]
- 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 yellow answer sheet**.

Experimental Tasks



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6. Continue adding 0.1 ml of sodium carbonate solution and write the pH values in **Table B.1 in the yellow answer sheet**, till the pH of the solution reaches 10. Also write the total volume of sodium carbonate solution added.

[B.Q2: 1.0 mark]

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 yellow answer sheet**.

[B.Q1.C: 0.25 marks]

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 yellow answer sheet**.
11. Continue adding 0.1ml of acetic acid solution and write the pH values **in Table B.1 in the yellow answer sheet**, till the pH of the solution reaches 4. Also write the total volume of acetic acid solution added.

[B.Q2. 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 yellow answer sheet**.

[B.Q1.D: 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 yellow 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 yellow answer sheet**. Also write the total volume of sodium carbonate solution added.

[B.Q3: 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.

Experimental Tasks



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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 yellow answer sheet**. Also write the total volume of acetic acid solution added.

[B.Q3: 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 yellow answer sheet** whether the following two statements are true (T) or false (F).

- 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.

[B.Q4: 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:

- 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 yellow answer sheet**.

[B.Q5: 1.0 mark]

B2 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.

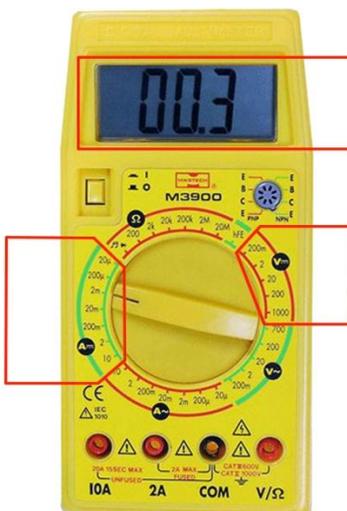
You are supplied with the following:

	Labeled as...	Quantity Supplied
Power supply; 500 mA, 3 V		1
Acrylic set-up with photodiode (see photo on page 9)		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		

Note: The white LED has a white base. The blue LED has a coloured base.

The photo below is that of a multimeter. Your multimeter may be either yellow or black.

Multimeter knob is to be positioned in this region for current measurement
2 μ A is the minimum range of current measurement
10A is the maximum range



If display read -1, it implies range is insufficient. Shift the knob to higher range. Working in higher range than required will result in loss of accuracy

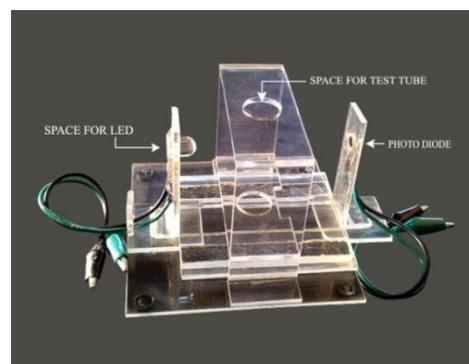
For this experiment DO NOT keep the knob in A ~V ~ Ω

Multimeter knob is to be in this region for voltage measurement



1. Press MODE button, if the Stopwatch is on Time Mode, to bring it to Stop Watch Mode. 0:00 00
2. Press SPLIT/RESET button to reset the Stopwatch to Zero.
3. Press START/STOP button to start the Stopwatch.
4. Press START/STOP button to stop the Stopwatch.
5. In Stopwatch mode START/STOP button should be used to start and stop as many times as required.

Stopwatch



Acrylic set-up with photodiode

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.
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.
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 yellow answer sheet.**

[B.Q6.A: 0.5 mark]

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

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 yellow answer sheet.**

[B.Q6.B: 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).
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 yellow answer sheet.**

Experimental Tasks



Time : 3 hrs
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14. Continue recording the values of current up to 7 minutes.

[B.Q7: 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.

[B.Q8: 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.

[B.Q9: 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.

[B.Q10: 1.0 mark]

B3 Estimation of calcium content in milk

Calcium content in milk can be estimated by a special form of titration using a reagent called **Na₂EDTA**. Na₂EDTA 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 ₂ EDTA solution (0.0027 M)	EDTA	80 ml in plastic bottle
Funnel		1

Procedure:

1. Add the Na₂EDTA solution to the burette using the funnel.
2. Write the initial burette reading in **Table B.4 in the yellow 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).

Experimental Tasks



Time : 3 hrs
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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 yellow answer sheet**
11. Repeat the titrations twice.
12. Enter your readings in the observation **Table B.4 in the yellow 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 yellow answer sheet.**
14. Calculate the average volume.

[B.Q11: 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).

[B.Q12: 1.0 mark]



Experimental Tasks



Time : 3 hrs
Marks :40

Space for rough work