A Course based Project Report On

DETERMINATION OF CALCIUM CONTENT IN DIFFERENT MILK SAMPLES

Submitted in partial fulfilment of
requirement for the completion of the
Engineering Chemistry Laboratory course.

B. Tech

ELECTRONICS AND INSTRUMENTATION ENGINEERING

of

VNRVJIET

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DECLARATION

We hereby declare that this Project Report titled **"DETERMINATION OF CALCIUM CONTENT IN DIFFERENT MILK SAMPLES"** submitted by us of **Electronics and instrumentation engineering** in**VNR Vignana Jyothi Institute of Engineering and Technology,** is a bonafide work undertaken by us and it is not submitted for any other certificate/course or published any time before.

Signature of the Student/Date

1.

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CERTIFICATE

This is to certify that the project entitled **"DETERMINATION OF CALCIUM CONTENT IN DIFFERENT MILK SAMPLES"** submitted in partial fulfilment for the course of Engineering Chemistry Laboratorybeing offered for the award of B.Tech (**EIE-A**) by **VNR VJIET** is a result of the bonafide work carriedout by **23071A1051,23071A1052,23071A1058,23071A1060**during the year **2023-2024**. This has not been submitted for any other certificate or course.

Internal Examiner **External Examiner**

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ABSTRACT

Milk is primary source of nutrition. It contains dissolved carbohydrates, proteins, vitamins, fats, minerals like Calcium, Magnesium, and Sodium. Calcium is the most abundant mineral in human body. It is important for biological processes. The aim of the present study was to determine the amount of calcium in milk samples from different sources and the effect of heat on Calcium content. Milk samples were collected from Desi cow, Jersey cow, Goat, Buffalo and pasteurize milk. Amount of Calcium was determined by EDTA titration method. The amount of Calcium was highest in goat milk and lowest in pasteurized packaged milk.

INTRODUCTION

Milk is white liquid produced by mammary gland of mammals. It is primary source of nutrients for young mammals before they are able to digest other type of food (Islam et al., 2014a). India is the largest producer of milk throughout the world. In the world there are more than 6 billion consumers of milk and milk products. Over 750 million people live within dairy farming households. Milk is processed into variety of products such as cream, butter, cheese, yoghurt, ghee, curd, khoa, butter milk, paneer and ice cream, etc. Modern industrial processes use milk to produce casein, whey protein, lactose, condensed milk, powdered milk, and many other food additives and industrial products ("Milk," 2016). Milk has energy required for human activities and nutrients needed for building up the human body (Yoo et al., 2013).

Calcium in milk has been the subject of much research, ever since it was established as an important mineral in milk by Wright (1929). Its role in chemical reactions, its molecular arrangement with phosphorus, and its effect on heat coagulation of proteins were studied later by White and Davies (1958a, 1958b, 1958c, 1958d), Pyne and McGann (1960), Pyne and Ryan (1950), Morrissey (1969), Fox and Hoynes (1975), van Boekel et al. (1989) and Holt (1992). Calcium is a very important component of all mammalian milks. It provides much of the dietary calcium requirement and plays an important role in the stability of the casein micelle. It is present in variable amounts in milks of different species (mM): cow 30.0; goat 25.0, sheep 42.0 (McCance and Widdowson 1988) It is thought to be related to how fast the animal grows; values are high in dogs and horses and lower in primates and humans. It can range from less than 10 mM in human milk to as high as 100 mM in rabbit milk (Holt 1981), on average, cow's milk contains approximately 30 mM of total calcium; about 20 mM is associated with the casein micelle and 10 mM is soluble calcium, mostly as undissociated calcium phosphate or citrate (Holt et al.1981). A small proportion of this is ionized and is termed ionic calcium (Ca^{2+}). At the normal pH of milk, less than 10% of the total calcium is present in its ionic form. It is the most abundant divalent cation found in milk; as such it counteracts the negative charge on the surface of the casein micelle. However, other cations might also be involved, such as H (pH), Na, K and divalent Mg.

2.1. Sources of milk

Sources of milk from domesticated animal include milk, not only of cattle but also of sheep, goat and buffalo (Imran et al., 2008). Other dairy cows include Ayrshire, Brown Swiss, Guernsey and Jersey. In Egypt, buffaloes are used mainly for milk production. In India, most of the people consume milk from cattle like Jersey and Buffalo. In developed countries, Dairy cattle such as Holstein have bred selectively for increased milk production. In the United States about 90% and in Great Britain about 85% of dairy cow are Holstein. Buffalo milk is pure white because it does not have carotene since carotene is processed into vitamin A. The main components of goat milk are similar to those of cow milk but differ as to particular physical and chemical properties. Goat milk proteins and fats have differences in their composition from the milk of other species. Goat milk was found to contain more of calcium and phosphorus than cow and human milk (Belewu et al., 2002).

2.2. Components of milk:

The composition of milk may change over a period of time and may vary from country to country. Milk contains water, carbohydrate, fats, protein, enzyme, vitamins, organic acids, and minerals like Phosphorus, Potassium and Calcium (Imran et al., 2008). Milk fat is secreted in the form of fat globule. Size of fat globule varies from 0.2 to15µm.It varies from species to species. Fat soluble vitamin A, D, E and K are present along with essential fatty acid in milk (Harding, et al., 1995). Milk contains Vitamin D which promotes calcium absorption in the gut and maintain adequate serum calcium and phosphate concentration to enable normal mineralization of bone, without sufficient Vitamin D, bone can become thin. Vitamin D prevents rickets in children and osteomalacia in adult. Important milk protein is casein, present in large amount. Milk also contains other proteins including enzymes. Milk proteins apart from casein are more water soluble. Milk also contains different carbohydrate like lactose, glucose and galactose. Lactose gives sweet taste to milk. Level of lactose depends on type of milk (Harding, et al., 1995). Milk contains various minerals like Calcium Magnesium, Sodium and Potassium. Mineral contribute to important physiological processes. High mineral content and availability of milk makes dairy products important source of minerals to human. They are important for maintaining blood pressure (Buitenhuis et al., 2015). The mineral content of goat milk is higher than that of cow milk. It also contains greater variety of mineral and is particularly rich in Iron, Phosphate and Potassium salts as compared to milk of other animals Calcium is the most important mineral in the human body. It is important for intracellular metabolism, bone growth, blood clotting, nerve conduction, muscle contraction, cardiac function, fertilization, heart rate, nerve conduction, stability of blood pressure, strength of bones and teeth. In mammals, oocyte maturation and egg activation at fertilization are controlled by changes in intracellular free Ca^{2+} level. Ca^{2+} regulates meiosis and fertilization Buffalo and goat milk provide great amount of Calcium. In Pakistan, the amount of calcium was found to be (644±76.8) mg/lit in goat, followed by cow (680±79.8) mg/lit and higher in buffalo milk (702±88.1) mg/lit.

2.3. Importance

- Calcium is an essential mineral for bone health, muscle function, nerve signaling, and other physiological processes. Milk is a significant dietary source of calcium for many people, especially children and adolescents whose bones are still developing. vital nutrient.
- Monitoring the calcium content in milk is crucial for quality control in the dairy industry. Consistent calcium levels ensure that consumers receive the expected nutritional benefits from the milk they purchase. It also reflects the quality of the milk production process.

2.4. EDTA

EDTA is used as a chelating agent to remove toxic heavy metals, like lead, mercury, and cadmium, from the body. This process, known as chelation therapy, is particularly important in cases of heavy metal poisoning or lead toxicity. EDTA binds to these metals in the bloodstream, forming a complex that can then be excreted from the body, helping to mitigate their toxic effects.

2.5. Calcium

Calcium plays a crucial role in muscle contraction and relaxation. When a nerve stimulates a muscle, calcium is released from storage in the muscle cells, allowing the muscle fibers to contract. Without sufficient calcium, muscle function can be impaired, leading to muscle weakness or cramping.

OBJECTIVES

- To determine the calcium content in milk samples using complexometric titration with EDTA
- To understand the importance of calcium in milk and its bioavailability
- To learn the principle of complexometric titration and its application in determining calcium content
- To analyze the calcium content in different milk samples and compare the results
- To understand the role of EDTA and Patton-Reeder indicator in the titration process
- To calculate the percentage of calcium in milk samples using the EDTA method

3.1. Keywords

1. Ligand
2. Chelating agent
3. Buffer
4. Indicator
5. Equivalence point
6. End point

3.2. Apparatus required

1. Weighing bottle
2. Volumetric flask
3. Beaker
4. Burette
5. Pipette
6. Conical flask
7. Wash bottle

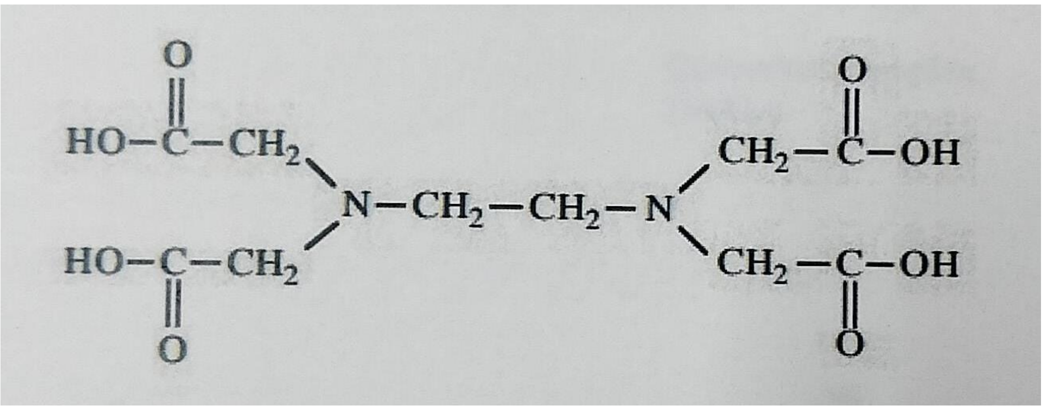
3.3. Reagents required

1. MgSO4.7H2O
2. Disodium salt of Ethylenediaminetetraacetic (EDTA)

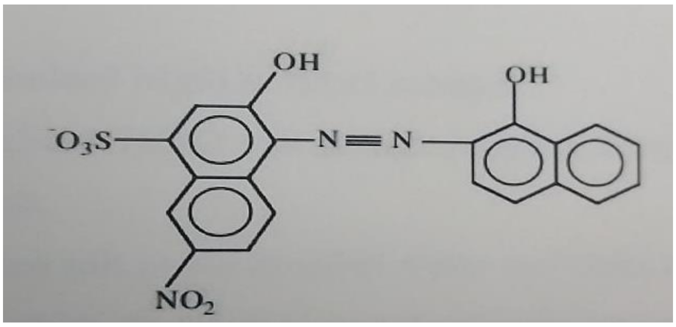
3. Eriochrome Black—T (EBT) Indicator
4. Buffer (NH4OH+NH4Cl)

3.4. principle

The classic method of determining calcium and other suitable cations is titration with a standardized solution of ethylenediaminetetraacetic acid(EDTA).EDTA has the structure shown below[3].



The endpoint of an EDTA titration is determined with a metallochromic indicator. These indicators are complexing agents that change color when combined with metal ions. A variety of indicators can be used for EDTA titrations. In this experiment, we will use Eriochrome black T (EBT) indicator, having the structure shown below



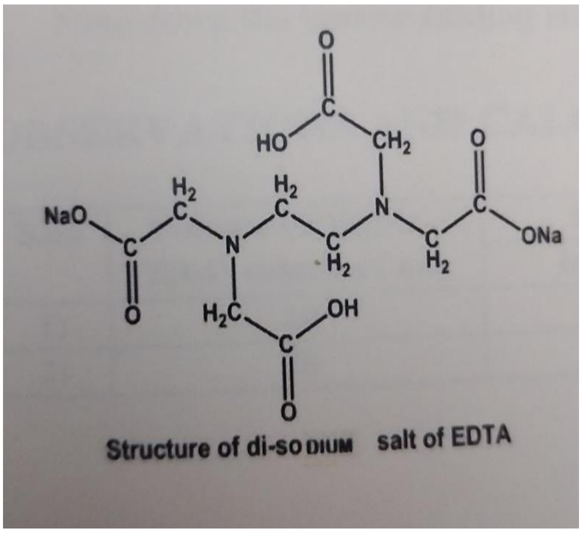
The determination of calcium in milk is based on a complexometric titration of calcium with aqueous solution of disodium salt of EDTA at high pH value. Addition of Eriochrome Black-T indicator to the milk at pH range 9-10 forms purple coloured unstable complex with Ca ions of the sample milk. The unstable purple colour complex forms a colourless stable complex with EDTA and liberates the blue coloured EBT indicator.

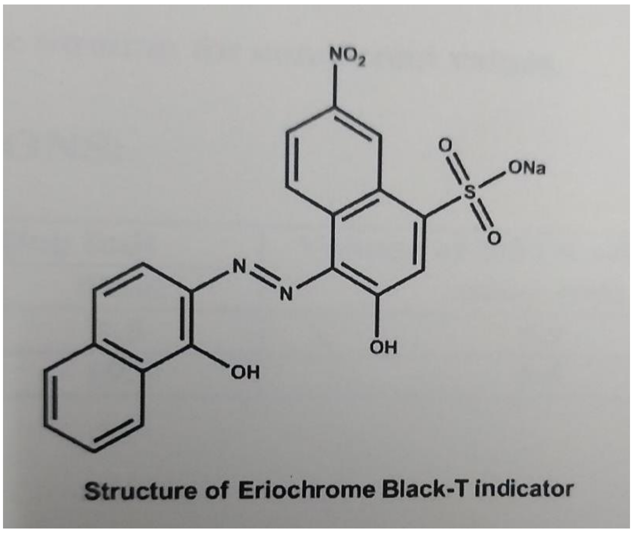
Ca^{2+} EBT ⟶ [Ca-EBT]

Purple Colour Complex (Unstable)

[Ca-EBT] +EDTA ⟶ [Ca-EDTA] + EBT

Colourless complex stable) Blue





PROCEDURE

Step-1: Preparation of standard $MgSO_4 . 7H_2O$ solution

- Weigh the given $MgSO_4.7H_2O$ salt using weighing balance and transfer into a clean 100ml volumetric flask.
- Dissolve the transferred salt in the distilled water and make up the solution up to the mark.
- Shake the volumetric flask for a while to get uniform concentration.

Calculations:

Weight of $MgSO_4$ 7H:O salt transferred (W)= 0.134 g

Normality of $MgSO_4.7H_2O$ = Weight of salt* 1000 /Equivalent Weight of Salt* 100

(Equivalent Weight of $MgSO_4.7H_2O$ is 123)

= (0.134/123) * 10

= 0.0108 N

Step-2: Standardization of EDTA solution

- Pipette out 20ml of Standard $MgSO_4.7H_2O$ solution into a clean 250 ml conical flask.
- Add 2 ml of buffer (NH_4OH+NH_4Cl) to maintain pH between 9-10.
- Add 2-3 drops of Eriochrome Black-T indicator, the solution then turns to purple.
- Fill the burette with EDTA solution and adjust to zero, then fix it to the burette stand.
- Now titrate the above $MgSO_4.7H_2O$ solution against EDTA solution till the purple solution turns to blue which is the end point of the titration.
- Note down the burette reading and repeat the titration for concurrent values.

4.1.&4.2.observations and calculations

s.no	Volume of $MgSO_4.7H_2O$ Solution(ml)	Burette reading(ml)		Volume of EDTA solution run down(ml)
		Initial	Final	
1	20	0	10.5	10.5
2	20	10.5	21	10.5

$N_1V_1 = N_2V_2$

N_1 = Normality of $MgSO_4.7H_2O$ solution= 0.01 N

V_1 = Volume of $MgSO_4.7H_2O$ solution = 20 ml

N_2 = Normality of EDTA solution=?

V_2 = volume of EDTA solution =10.5 ml

Normality of EDTA solution (N_2)=N_1V_1/V_2

$= (0.01 * 20) / 10.5 = 0.019N$

step 3: preparation of milk samples

- Take 5 ml of the milk sample and transfer into a clean 100 ml volumetric flask.
- Add distilled water to the milk sample and make up the solution up to the mark.
- Shake the volumetric flask for a while to get uniform concentration.

step-4: estimation of calcium content in milk

- Pipette out 20ml milk sample-1 into a clean 250 ml conical flask.
- Add 2 ml of buffer (NH_4OH+NHCI) to maintain pH between 9-10.
- Add 2-3 drops of Eriochrome Black-T indicator then solution turns to purple in colour.
- Fill the burette with EDTA solution and adjust to zero then fix it into the burette stand.

- Now titrate the above milk sample against EDTA solution till the purple solution turns to blue which is the end point of the titration.
- Note down the burette reading and repeat the titration with milk samples 2,3

Sample	s.no	Volume of MgSO4.7H2O Solution(ml)	Burette reading(ml)		Volume of EDTA solution run down(ml)
			Initial	Final	
Toned milk	1	20	0	3.3	3.3
	2	20	3.3	6.6	3.3
Jersey standardized milk	1	20	7	10.1	3.1
	2	20	11	14.1	3.1
Jersey full cream milk	1	20	11	14.5	3.5
	2	20	14.5	18	3.5

Calcium content (in mg/L) =

$$\frac{\text{(Volume of EDTA used in titration*Calcium concentration of EDTA solution*Dilution Factor)}}{\text{Volume of Milk Sample taken}}$$

Calcium content in:

Toned milk = $\frac{\text{Normality of EDTA Volume of EDTA *40.78 * 1000}}{\text{Volume of milk sample taken}}$

= (0.019*3.3*40.78* 1000)/20=127.84ppm

Jersey standardized milk = $\frac{\text{Normality of EDTA Volume of EDTA*40.78*1000}}{\text{Volume of milk sample taken}}$

= (0.019*3.1*40.78 *1000)/20=120.0971 ppm

Jersey full cream milk = $\frac{\text{Normality of EDTA *Volume of EDTA* 40.78 *1000}}{\text{Volume of milk sample taken}}$

= (0.019* 3.5* 40.78* 1000)/20=135.5935ppm

4.3. result

Sample / Milk content	Toned milk	Jersey standardized milk	Jersey full cream milk
5%	127.84	120.0971	135.5935

4.4. discussion

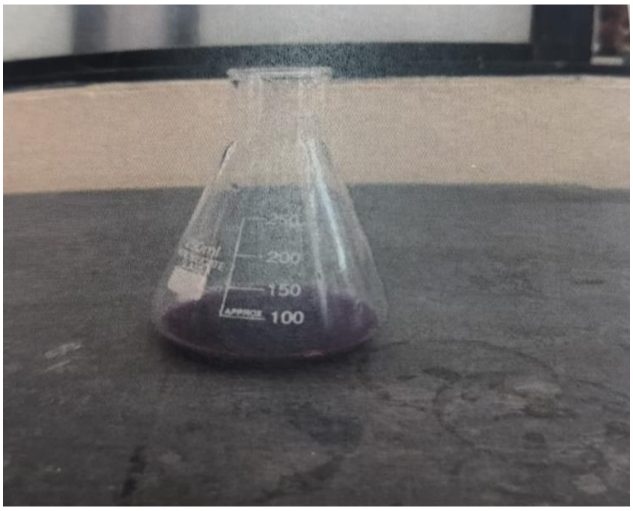
At the beginning of the study, some experiments were carried out for the determination of the effect of calcium as an activator on the catalase enzyme biosensor. For this purpose firstly, the developed biosensor was used only for hydrogen peroxide detection using standards with concentration between 1 and 10 m Min the absence of calcium and a linear curve was obtained. After that, by using the same hydrogen peroxide standards but in the presence of 5 mM calcium a new standard curve was obtained. Fig. 1 shows the results obtained from the experiments. According to the figure the biosensor responses increased very efficiently in the presence of calcium


DODLA
Toned Milk
Fat 3.0%


Godrej
Jersey
STANDARDIZED
MILK
Balance of taste
and thickness
Homogenized
& Pasteurized


Godrej
Jersey
RICH
FULL CREAM
MILK


35ML




250ml

CONCLUSION

Determining the calcium content in milk is a crucial aspect of assessing its nutritional value. Through the utilization of a titration method, the concentration of calcium ions in the milk sample was successfully determined. The titration involved the use of ethylenediaminetetraacetic acid (EDTA) as a complexometric titrant, forming a stable complex with calcium ions. The results obtained from the titration indicated that the milk sample contained a significant amount of calcium, an essential mineral for bone health and various physiological functions in the human body. The precision and accuracy of the titration method were enhanced through careful standardization of the EDTA solution The significance of determining calcium in milk lies in its implications for human health. Calcium plays a vital role in bone formation, blood clotting, muscle function, and nerve transmission. Therefore, accurate measurement of calcium content in milk allows consumers to make informed dietary choices and ensures the delivery of essential nutrients for overall well-being and meticulous titration techniques. In conclusion, the titration method employed in this study provided a reliable means of determining calcium concentration in milk. The results contribute valuable information to the broader understanding of milk's nutritional composition and aid in promoting awareness of the importance of calcium intake for maintaining optimal health. This analytical technique can be applied in quality control processes within the dairy industry and is valuable for both consumers and producers in ensuring the nutritional value of milk products [7].

FUTURE SCOPE

- **Method Optimization**: Continued refinement and optimization of the EDTA method can enhance its accuracy, sensitivity, and efficiency. This includes exploring variations in reagent concentrations, pH levels, and reaction conditions to improve the precision and reliability of calcium determination across diverse milk samples.

- **Automation and High-Throughput Analysis**: Integrating automation and robotics into the EDTA method can streamline sample processing and analysis, enabling high-throughput screening of large numbers of milk samples. This can expedite research efforts and facilitate routine quality control in dairy production facilities.

- **Multi-Element Analysis**: Expanding the scope of analysis beyond calcium to include other essential minerals and trace elements present in milk, such as magnesium, potassium, and zinc, using the same EDTA method. This comprehensive approach can provide valuable insights into the overall nutritional composition and quality of different milk samples.

- **Validation and Standardization**: Rigorous validation studies across various types of milk (e.g., cow, goat, sheep, plant-based alternatives) and processing methods (e.g., raw, pasteurized, fortified) are essential to establish the robustness and applicability of the EDTA method. Standardizing protocols and reference materials can ensure consistency and comparability of results across different laboratories and studies.

- **Environmental Impact Assessment**: Investigating the environmental impact of calcium determination in milk production, including the use of EDTA and its disposal, can contribute to sustainable practices in dairy farming and processing. Exploring eco-friendly alternatives or recycling strategies for EDTA waste can mitigate potential environmental risks.

- **Clinical and Nutritional Studies**: Conducting clinical trials and epidemiological studies to elucidate the relationship between calcium content in milk and human health outcomes, such as bone health, cardiovascular disease risk, and overall nutritional status. This research can inform dietary recommendations and public health policies regarding milk consumption and calcium intake.

- **Quality Assurance and Regulatory Compliance**: Implementing the EDTA method as a reliable tool for quality assurance and regulatory compliance in the dairy industry.

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