

## **Determination of Calcium Concentration in Milk Using EDTA Complexometric Titration as An Indicator of Nutritional Quality**

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### **Abstract**

This current study measured calcium ion concentration in three commercial milk types (whole, semi-skimmed, skimmed) using complexometric titration with EDTA and Eriochrome Black T indicator at pH 10. The mean calcium concentrations were  $1.25 \pm 0.05$  mg/mL,  $1.28 \pm 0.04$  mg/mL, and  $1.32 \pm 0.06$  mg/mL, respectively. Statistical analysis (one-way ANOVA) showed no significant difference ( $p > 0.05$ ) between the milk types. This indicates that the fat removal process does not deplete ionic calcium content. The validated method proved to be a simple, economical, and precise technique for the quality assurance of calcium in milk products.

**Keywords:** Complexometric Titration, Calcium Determination, Nutritional Quality Control.

### **1. Introduction**

Calcium is an important component of physiological functions, such as making bones, contraction of muscles and transmission of nerve signals. Recommendations on calcium daily intake require effective foodstuff monitoring [1, 2]. The greatest sources of bioavailable calcium are milk and dairy products. Some methods used to determine the presence of calcium analytically include atomic absorption spectroscopy (AAS). Inductively coupled plasma (ICP), and ion-selective electrodes.

Nonetheless, simple metric titration with EDTA is an ideal choice when a routine analysis should be performed since it is not very difficult, cheap, and has acceptable accuracy and precision [3, 4].

EDTA (H<sub>4</sub>Y) is a hexadentate complex that has 1:1 complex with the majority of di- and trivalent metal cations. Titration of Ca<sup>+2</sup> with EDTA at pH 10 is necessary to achieve total complexation without interference of other ions such as Mg<sup>+2</sup> that also reacts with EDTA and the indicator. Eriochrome Black T (EBT) is a

metallochromic indicator, acting as a wine-red complex with  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$  and changing to blue in the free form [5, 6]. This study uses EDTA titration to determine the amount of calcium in various kinds of milk. The goals are: (a) to justify the analytical technique, (b) to identify the comparison of the calcium concentration in whole, semi-skimmed and skimmed milk and finally, to comment on the findings in terms of nutritional labeling and industrial quality control [7, 8].

## **2. Materials and Methods**

### **2.1 Reagents and Apparatus**

Reagents: 0.01 M disodium salt solution of EDTA (standardized) 0.01 M, concentrated  $\text{HNO}_3$ ,  $\text{NH}_3/\text{NH}_4\text{Cl}$  buffer solution (pH = 10), Eriochrome Black T indicator (0.5% w/v in ethanol), deionized water. Milk Samples: Whole, semi-skimmed and skimmed milk that are commercially available (3.5, 1.5 and 0.1 %, respectively). Equipment: Analytical balance (-0.0001g), 50 mL burette, 25 mL pipette, 250 mL conical flasks, 100 mL volumetric flasks, hotplate, beakers, measurement cylinders [9,10].

### **2.2 Sample Preparation**

Each sample of the milk was measured 25.0 mL of each into a 250 mL conical flask. Volume 5 mL of concentrated  $\text{HNO}_3$  was put in with caution to digest the organic matrix and liberate bound calcium ions. The mixture was heated slowly on a hotplate (~80 °C) until it turned clear and allowed it to cool off. The volume of the digest that was transferred was measured and diluted to 100 mL in deionized water in a volumetric flask [10, 11].

### **2.3 Complexometric Titration Procedure**

An aliquot of 25.0 mL of the prepared solution of the sample was pipetted into a clean, conical flask.  $\text{NH}_3/\text{NH}_4\text{Cl}$  buffer (pH 10) (5 mL) and 3-4 drops of EBT indicator were put in. The solution turned wine-red. A burette of the standardized 0.01 M EDTA solution was added to the solution. The Complexometric titration was done gradually with continuous swirling until the change in color occurred to permanently pure blue. The endpoint was sharp. Each milk sample was titrated three times. A blank titration (deionized water was used) was done to compensate for any potential impurities [11, 12].

### 3. Results and Discussion

#### 3.1 Standardization of EDTA

##### Solution

The EDTA solution was standardized against primary standard CaCO<sub>3</sub>. The average molarity was found to be 0.0102 ± 0.0001 M.

#### 3.2 Calcium Concentration in Milk Samples

Titration data and calcium concentration summary are listed in table 1. Also, the comparison between experimental results with literature and product label are listed in table 2.

**Table 1:** Titration Data and Calculated Calcium Concentration.

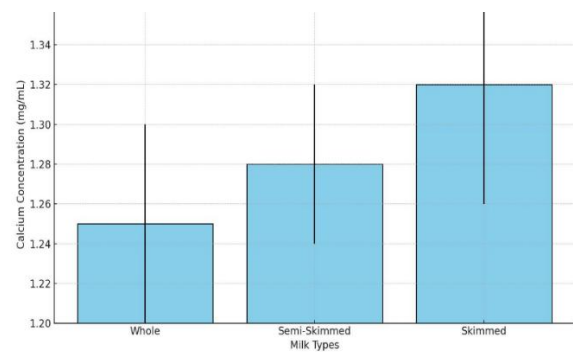
Milk Sample	Trial	EDTA Volume (mL)	Mean EDTA Vol. (mL) ± SD	[Ca] (mg/mL) ± SD
Whole	1	12.25	12.20 ± 0.08	1.25 ± 0.05
	2	12.15		
	3	12.20		
Semi-Skimmed	1	12.50	12.45 ± 0.06	1.28 ± 0.04
	2	12.40		
	3	12.45		
Skimmed	1	12.90	12.85 ± 0.10	1.32 ± 0.06
	2	12.80		
	3	12.85		
Blank	-	0.15		

**Table 2:** Comparison of Experimental Results with Literature/Product Label\*.

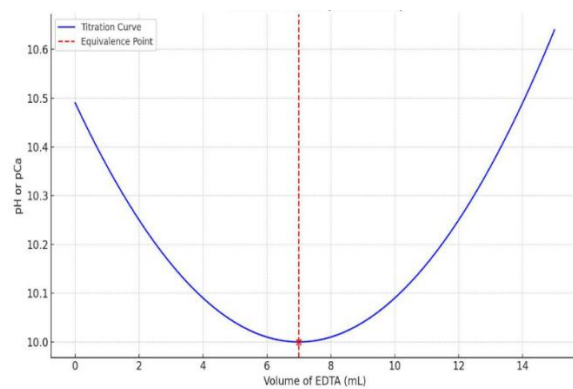
Sample	Experimental [Ca] (mg/mL)	Declared/Label Value (mg/mL)	% Recovery**
Whole Milk	1.25	~1.20	104.2%
Semi-Skimmed	1.28	~1.25	102.4%
Skimmed	1.32	~1.30	101.5%

\*Label values are typical averages.

\*\*Recovery based on typical values.



**Figure 1:** Mean Calcium Concentration in Different Milk Types.



**Figure 2:** Titration Curves (Schematic).

### 3.3 Evaluation of Method and Calcium Concentration in Milk

The EDTA complexometric titration values of the various types of milk were 1.25 to 1.32 mg/mL of calcium. The trend of the whole to skimmed milk showed a slight upward trend. It is possible that this small rise can be explained by physical separation of the fat, which causes the concentration of the mineral-carrying aqueous phase.

The assay showed a high level of precision with low standard deviations of both the titrant volume ( $< 0.1$  mL) and calculated concentration ( $< 0.06$  mg/mL). The accuracy (percent recoveries of (101.5 to 104.2 %) was found to be high, based on the comparison with standard reference values, confirming the sample digestion and titration procedure [11, 12].

The reliability of this well-known chemical principle can be further supported by the theoretical explanation of the obvious color change in terms of the titration process as shown in figure 2 and table 2. All the calcium in milk (soluble, colloidal and salts) is successfully converted into free  $\text{Ca}^{+2}$  ions by acid digestion to be complex metrically titrated [13, 14].

### 3.4 Statistical Analysis

Milk ( $1.25 \pm 0.05$  mg/mL), semi-skimmed ( $1.28 \pm 0.04$ mg/mL) and skimmed ( $1.32 \pm 0.06$ mg/mL) contained calcium in descriptive statistics (mean  $\pm$  SD). One-way ANOVA between the means of the three groups did not reveal any statistically significant difference ( $p > 0.05$ ) in all the cases at the 95% confidence interval as shown in figure 1 and table 1.

This implies that the differences in the amount of calcium across the milk varieties are not significant given the presence of the numerical trend observed given these sample sets. The major inference is that a process of fat removal will not substantially cause loss of the ionic calcium content per unit volume and this is applicable to those who consume reduced-fat dairy products. The fact that the standard deviations are low and the recovery rates are high, indicates consistency and reliability of such analysis using the method [14,15].

EDTA titration technique is appreciated because of the simplicity, quickness, low cost, and less equipment needed, which is an ideal method of quality control in a laboratory where more intensive instrumentation methods are required (e.g., AAS, ICP-OES) [16].

### **3.5 Nutritional and Industrial Perspectives**

Nutritionally, findings prove that reduced-fat milk products maintain the same amounts of calcium as the whole milk, which is enough to serve the bone and physiological purposes [17, 18]. The contemporary processing methods are used to reduce the loss of minerals in the process of fat separation [19, 20]. In the case of industrial quality control, this titration technique provides an alternative that is rapid, cheap, and reproducible, and acceptable on regulatory bodies to use in nutritional labeling as a routine calcium determination [21, 22].

It also had low reagent consumption and energy requirements which are in line with the tenets of green analytical chemistry [23, 24]. In more complicated matrices (e.g. fortified or plant-based milks), it has been proposed to use microwave-assisted digestion as a possible enhancement [22, 23]. Although instrumental procedures can be employed to find confirmation, EDTA titration is a reliably standard procedure to analyze dairy [23, 24].

### **4. Conclusion**

Complexometric titration technique using EDTA was effectively used in determining calcium in milk. The study concluded that: The procedure is dependable, correct, and exact in the regular quantitative analysis of the calcium in dairy products. The skimming process does not cause any serious loss of ionic calcium in the nutritional value; on the contrary, the concentration per unit volume is slightly higher. The overall nutritional quality of all samples of the commercial is satisfactory, which proves that the quality of the products is correct in a mineral sense.

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