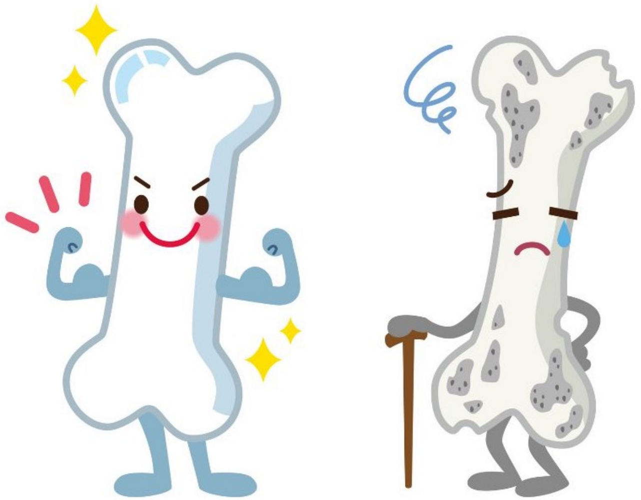
BCH 445- Biochemistry of Nutrition [Practical]

Lab (9) Determination of Calcium in Milk

Calcium

- **Calcium** is an important component of a healthy diet and a mineral necessary for life.
- **Calcium** is the most abundant mineral in the human body, making up 1.5 to 2% of the total body weight.
- It is a mineral that people need to build and maintain strong bones and teeth.
- It is also very important for other physical functions, such as muscle control and blood circulation.
- If we do not have enough calcium in our diets to keep our bodies functioning, calcium is removed from where it is stored in our bones.
- Overtime, this causes our bones to grow weaker and may lead to **osteoporosis** (a disorder in which bones become very fragile).



Milk and Calcium

- **Milk** is a heterogeneous mixture of proteins, sugar, fat, vitamins and minerals.
- Milk and milk products are some of the natural sources of calcium.
- The calcium concentration in bovine milk is about 1g/L.
- Cow's milk has good bioavailability of calcium (21 to 45%).
- Milk is an excellent source of dietary calcium for those whose bodies tolerate it because it has a high concentration of calcium and the calcium in milk is excellently absorbed.
- In the United States, approximately 72% of calcium intakes come from dairy products and foods with added dairy ingredients.

Practical Part

Objective:

- Determination of Calcium in milk sample.

Principle

- In this experiment, The determination of calcium in milk is based on a complexometric titration of calcium with an aqueous solution of the disodium salt of EDTA at high pH value=12.

- **Complexometric titration** is a type of titration based on complex formation between the analyte and titrant.

- Such compounds (**EDTA**) are capable of forming chelate complex with many cations (metal ion) in which the cation is bound in a ring structure.

- The ring results from the formation of a salt-like bond between the cation and the carboxyl groups together with a coordinate bond through the lone pair of electrons of the nitrogen atom.

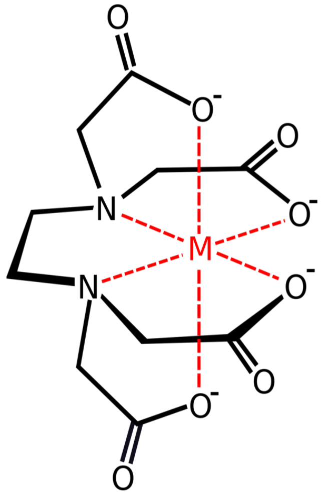


Figure 1. EDTA complex with metal ion

Principle

- The common form of the agent is **disodium salt Na_2H_2EDTA.**
- It is colorless and can be weighed and dissolve in water to form a stable solution.
- At high pH (>10) the remaining protons leave EDTA forming $EDTA^{4-}$ anion.

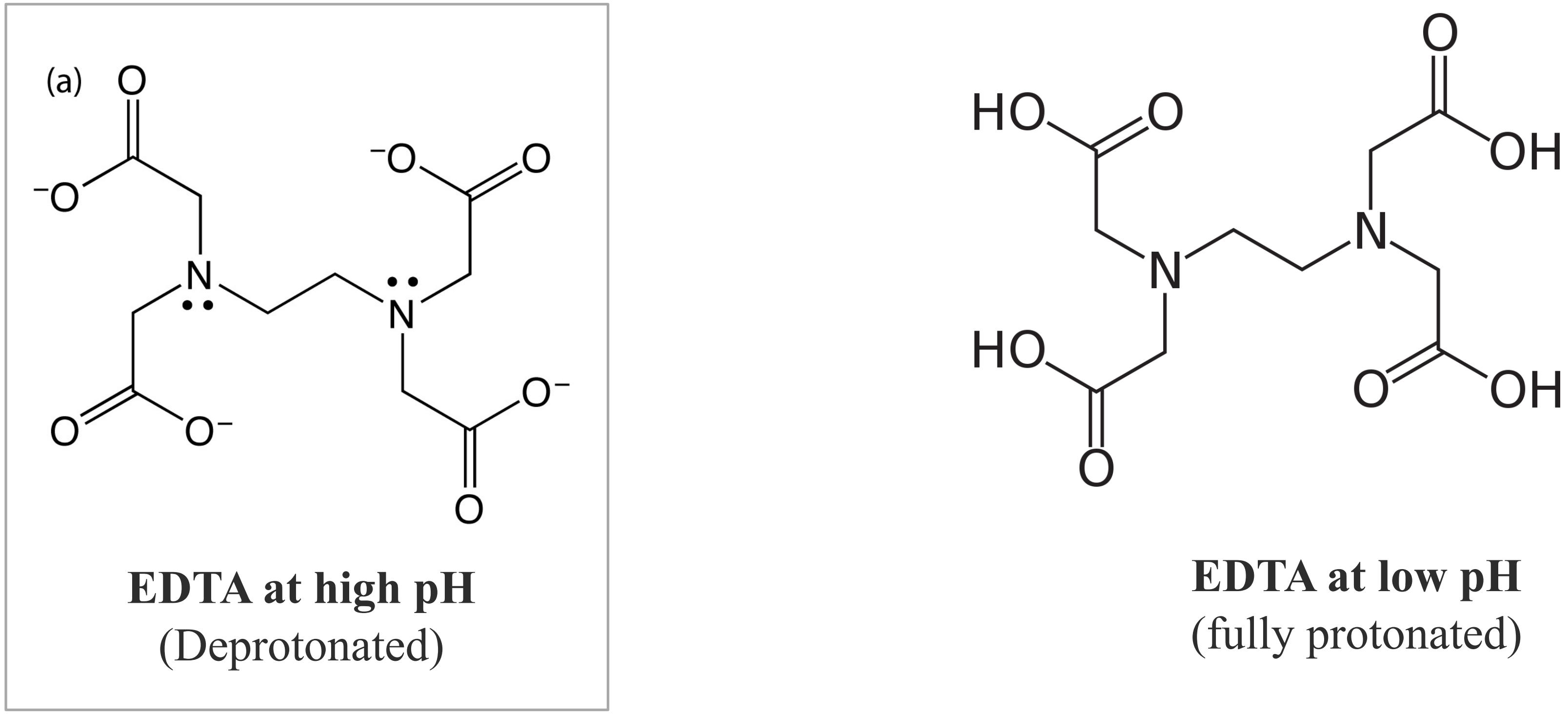


Figure 2. Protonated and deprotonate form of EDTA

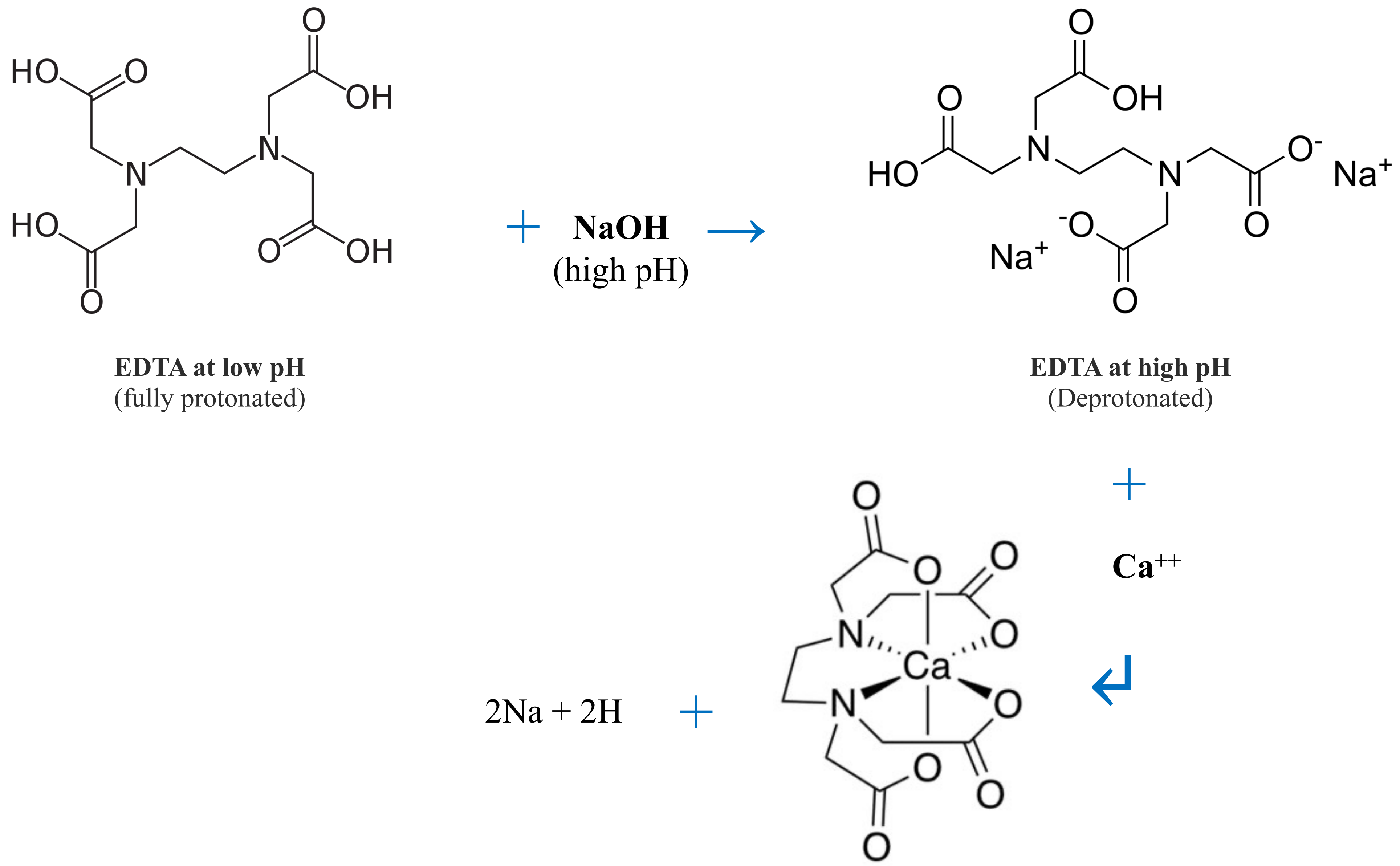


Figure 3. EDTA reaction with calcium ion at a high pH

Solochrome indicator

- The Solochrome dark blue indicator is a suitable indicator in this case.
- The dye itself has a blue color.
- This blue dye also forms a complex with the calcium ions changing color from blue to pink/red in the process, but the dye–metal ion complex is less stable than the EDTA–metal ion complex.
- As a result, when the calcium ion–dye complex is titrated with EDTA the Ca^{2+} ions react to form a stronger complex with the EDTA changing the dye color to blue.

1. Ca^{++} (colorless) + Indicator ➔ Ca-Indicator
2. Ca-Indicator + $EDTA^{4-}$ ➔ $Ca\text{-}EDTA^{2-}$ + Indicator

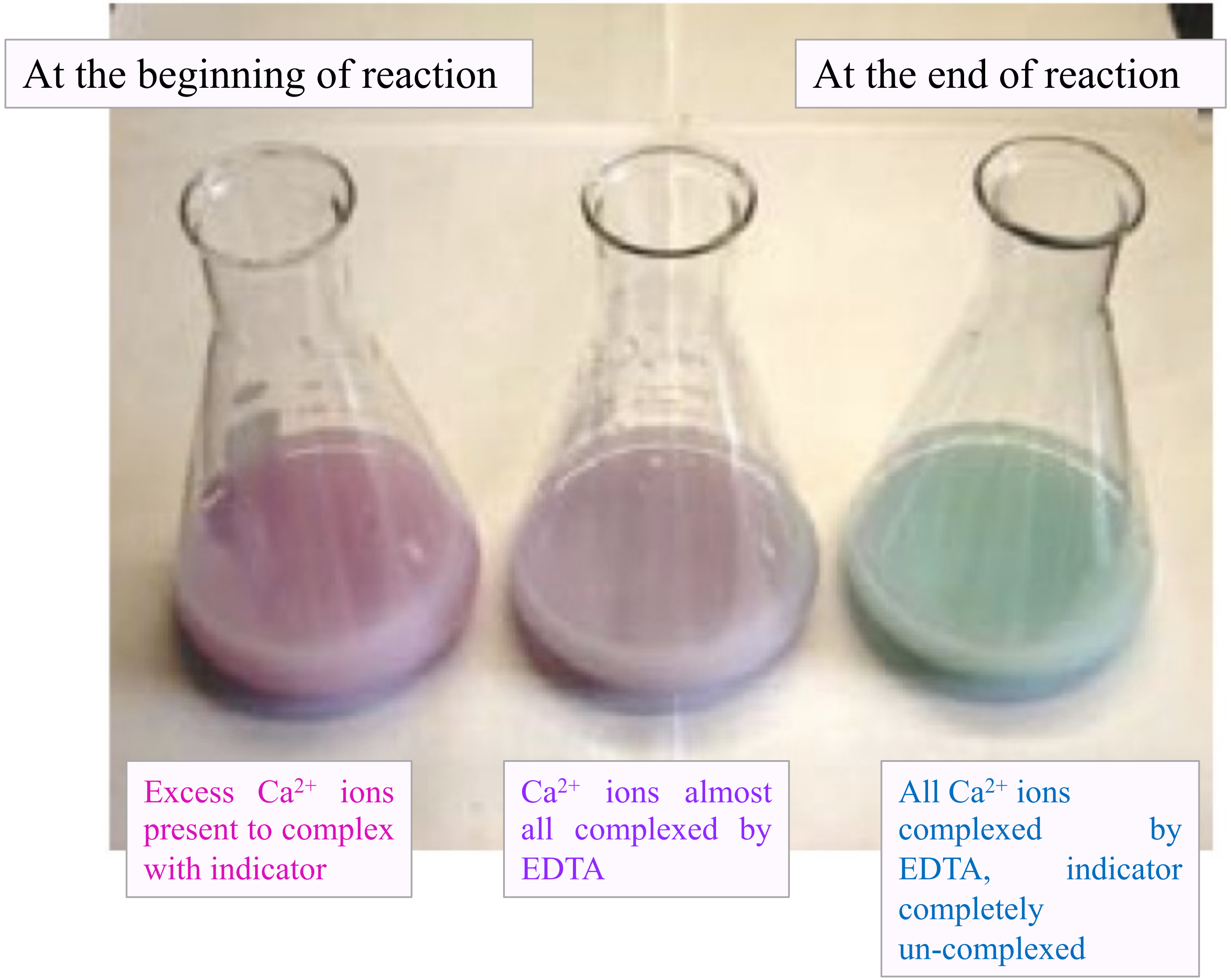


Figure 4. Color changes for calcium-EDTA titration

How to determine calcium in the presence of Mg?

- This method for determining **Ca^{2+}** concentration in the presence of **Mg^{2+}** relies on the fact that the pH of the solution is sufficiently high ((The pH will be approximately 12.5 due to the addition of concentrated NaOH solution)) to ensure that all magnesium ions precipitate as magnesium hydroxide before the indicator is added.
- In this condition, magnesium ions are precipitated as hydroxide and do not interfere with the determination of calcium.

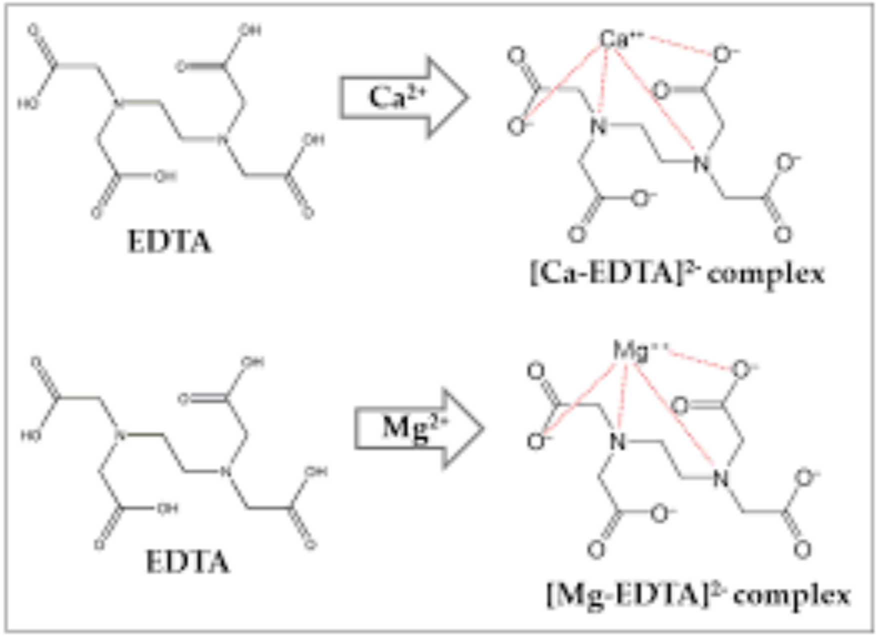


Figure 5. Calcium and magnesium complex with EDTA

Method

1. Combine 10mL of sample, 40mL distilled water, and 4mL of 8M sodium hydroxide solution into an Erlenmeyer flask and allow solution to stand for about 5 minutes with occasional swirling.
2. A small of magnesium hydroxide may precipitate during this time. Do not add the indicator until you have given this precipitate a chance to form.
3. Then add 6 drops of the Solochrome dark blue solution.
4. After that start to titrate with EDTA solution.
5. Repeat titration for three trials.

Results

	EDTA volume (ml)
1	
2	
3	
Average	

$$\textbf{Molarity (M)} = \frac{\text{number of moles}}{\text{liters of solution}}$$

$$\textbf{Number of moles (n)} = \frac{\text{Weight of substance}}{\text{molecular weight}}$$

Calculation

1. Calculate the moles of EDTA required to complex the Ca2+ ions in the sample:

➔Number of moles (for EDTA) = Molarity of EDTA x volume of EDTA in L

Note : Ratio Ca^{2+}:EDTA = 1 : 1 (i.e moles of EDTA = moles of Ca2+)

Molarity of EDTA = 0.03408 M

1. Calculate weight of Ca2+ :

➔Weight of Ca^{2+} = Number of moles x molecular weight (40.78)

- % of Ca2+ = (weight of Ca^{2+} / weight of sample) x 100

OR

- **CA^{2+} %=** $\dfrac{\text{[Molarity of EDTA X vol. of EDTA (in liter)]X 40.78) x 100}}{\text{Weight of the sample}}$

* 40.78 g/ mol is the molecular weight of Ca^{2+}

Additional resources

- https://www.youtube.com/watch?v=AiWFA-Bvg60
- https://www.youtube.com/watch?app=desktop&v=hTy9JBllUVg