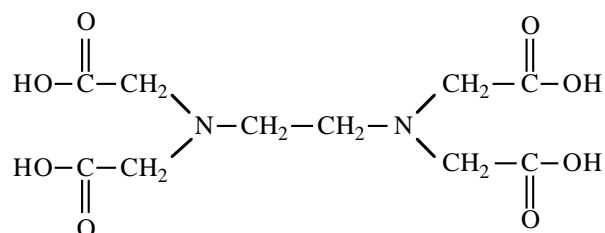


## Determination of calcium by Standardized EDTA Solution

### Introduction

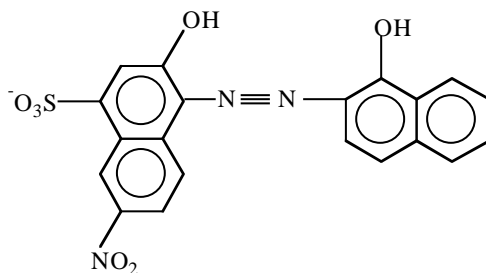
The classic method of determining calcium and other suitable cations is titration with a standardized solution of ethylenediaminetetraacetic acid (EDTA). EDTA has the structure shown below. Instead of repeatedly drawing this structure or writing out the chemical formula, the EDTA molecule is represented as "H<sub>4</sub>Y". Each acid hydrogen on EDTA can be removed, producing H<sub>3</sub>Y<sup>-1</sup>, H<sub>2</sub>Y<sup>-2</sup>, HY<sup>-3</sup>, and Y<sup>-4</sup> ions. The disodium dihydrate of EDTA, Na<sub>2</sub>H<sub>2</sub>Y•2H<sub>2</sub>O is commonly used to prepare standard EDTA solutions.



This salt is readily available from many commercial sources, and often in such a high purity that solutions need not be standardized for routine work. Primary standard calcium carbonate can be used to standardize EDTA solutions.

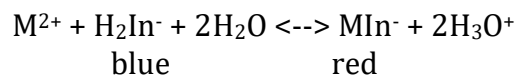
Of the various EDTA species, only the Y<sup>4-</sup> ion (the completely deprotonated anion of EDTA) forms a 1:1 complex with metal ions. To increase the fraction of Y<sup>4-</sup>, the pH needs to be increased to 10 in this experiment.

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.

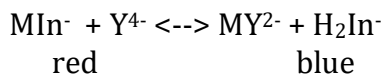


(H<sub>2</sub>In<sup>-</sup>)

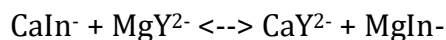
This indicator (shown as H<sub>2</sub>In<sup>-</sup> in the equations below) changes from blue to red when combined with a metal ion, forming a complex ion:



EDTA is a stronger complexing agent than the indicator, and displaces the indicator from the metal ion allowing the indicator to return (through shades of violet) to a pure blue color, indicating the end of the reaction.



Calcium ion ( $\text{Ca}^{2+}$ ) does not form a stable red complex with the EBT indicator; therefore the direct titration of  $\text{Ca}^{2+}$  by EDTA may not cause a sharp color change of EBT indicator at the end point. The magnesium complex with EBT is stable and the  $K_f$  of  $\text{Mg}^{2+}$  with EDTA is lower than the  $K_f$  of  $\text{Ca}^{2+}$  with EDTA. Thus, a displacement titration of  $\text{Ca}^{2+}$  by the mixture of  $\text{Mg}^{2+}$  and EDTA will help to determine the end point with the following mechanism:



To accomplish this displacement titration, a small amount of  $\text{Mg}^{2+}$  will be mixed with the EDTA solution. The EDTA-Mg mixture will titrate the unknown  $\text{Ca}^{2+}$  solution. At the end point,  $\text{Mg}^{2+}$  will be released from the EBT indicator and complexed with EDTA, causing the color change from red to blue.

Solutions needed for this experiment:

Solutions prepared by the student	Solutions provided by the instructor
0.01M disodium EDTA with $\text{MgCl}_2$ (standardized by student)	12M Hydrochloric acid
Standard $\text{Ca}^{2+}$ solution	8.5M $\text{NH}_3$ - $\text{NH}_4\text{Cl}$ Buffer
Solution of egg shells	Eriochrome black T indicator
5% Sodium Hydroxide	

## Experimental Procedure

Preparation and standardization of 0.01 M EDTA solution.

1. Using the top loading balance, weigh between 3.6 and 3.7 grams of disodium EDTA dihydrate into a clean 1 L plastic bottle. EDTA will leach metal ions from soft glass containers, and should never be stored in glass containers. Add 1 L of deionized water.

EDTA dissolves **SLOWLY**. Shaking or stirring the solution vigorously speeds the dissolution process. Nevertheless, even under these conditions EDTA dissolves **SLOWLY**. It is strongly recommended that the EDTA solution be prepared several hours or even the day before you plan on using it. Before use, check the solution to make sure all of the solid has dissolved.

2. Using the analytical balance, weigh out ~0.1 grams of  $\text{MgCl}_2$  and add the  $\text{MgCl}_2$  to the EDTA solution. You don't need to wait for the EDTA to dissolve before adding the magnesium chloride.
3. Dry about 1 gram of calcium carbonate ( $\text{CaCO}_3$ ) in the oven for 2 hours. Transfer to the desiccator and cool (~ 1 hour). When cooled, weigh a 0.5-gram portion of calcium carbonate on the analytical balance and transfer it to a clean 250 mL beaker.
4. Add approximately 25 mL of distilled  $\text{H}_2\text{O}$ , then 5 mL of conc. HCl **carefully** to the 250 mL beaker. Calcium carbonate reacts vigorously with acid, producing carbon dioxide gas, which may spatter the beaker contents. Cover the beaker with a watch glass. **Note:** If  $\text{CaCO}_3$  does not dissolve completely, add another 5 mL of conc. HCl. When the calcium carbonate has completely dissolved, boil the solution **gently** for 2-5 minutes, keeping the watch glass on the beaker, to expel carbon dioxide. Analytically transfer the solution to a 500.00 mL volumetric flask and QS with DI water.
5. Pipette 25.00 mL of standard  $\text{Ca}^{2+}$  solution prepared in step 4 into a 250-mL Erlenmeyer flask. Check the pH using pH paper: if acidic, use 5% sodium hydroxide solution to adjust the pH to ~7. When the pH is ~ 7, add 10 mL of 8.5M  $\text{NH}_3\text{-NH}_4\text{Cl}$  buffer. This buffer has been prepared for your use. **CAUTION! This buffer is dangerous; it is caustic and ammonia can cause pulmonary paralysis (it can interfere with your ability to breath). Exercise caution in handling and dispensing this buffer!!**
6. Add 20 mL of deionized water and 2-3 drops of EBT indicator. Titrate the  $\text{Ca}^{2+}$  standard solution with the EDTA solution until the color changes from wine red, through purple, to a pure rich blue color. At the end point, the last traces of purple in the solution will have just disappeared. If the reaction

seems to proceed slowly near the equivalence point, after each addition of EDTA wait a few seconds before adding the next drop.

Some students (not all of them male) have difficulty distinguishing between purple and blue. You may want to make a comparison sample, representing the endpoint of the titration. The comparison sample is made from 50 mL of DI water, 10 mL of 8.5M  $\text{NH}_3\text{-NH}_4\text{Cl}$  buffer, and 2 – 3 drops of EBT indicator solution. The solution should have a rich blue color and can be used for comparison with the end point of your calcium-EDTA titrations.

7. Perform the titration in triplicate. Normally, three titrations will agree within 0.04 mL. Do not use all of the EDTA solution in performing the standardization, since you must have enough EDTA to titrate the unknown  $\text{Ca}^{2+}$  and eggshell solutions.

From the known mass of  $\text{CaCO}_3$ , and the 1:1 stoichiometry between  $\text{Ca}^{2+}$  and EDTA, you should be able to readily calculate the molarity of the EDTA solution. Calculate the average molarity and label your EDTA solution appropriately.

Determination of the unknown  $\text{Ca}^{2+}$  concentration.

1. Pipette 5.00 mL of your unknown solution into 250.00 mL volumetric flask and QS with DI water. Pipette a 50.00 mL aliquot of the  $\text{Ca}^{2+}$  solution from the volumetric flask into a 250-mL Erlenmeyer flask. Adjust the pH to ~7 (if needed). Add 10 mL of 8.5M  $\text{NH}_3\text{-NH}_4\text{Cl}$  buffer, and 2-3 drops of EBT indicator to the Erlenmeyer flask, and titrate with EDTA. Perform this analysis in triplicate.

Determination of the percentage of  $\text{Ca}^{2+}$  in eggshell.

1. Peel off any membrane attached to your eggshell (the entire eggshell – not just a portion). You may need to carefully, thoroughly examine the eggshell to find all of the membrane. When the membrane has been removed, dry the eggshell in the oven, overnight, at 110 °C. Cool the eggshell in the desiccator, and weigh out a 0.2 g portion on the analytical balance. Dissolve the eggshell in 20 mL of 50% hydrochloric acid. Filter the solution to remove the organic solid in the solution.
2. Analytically transfer the filtered eggshell solution into a 250.00 mL volumetric flask and QS with DI water. Pipette a 25.00 mL aliquot of the solution from the volumetric flask into a 250.00 mL Erlenmeyer flask. Adjust the pH to 7 using dilute sodium hydroxide solution (if needed). Add 10 mL of 8.5M  $\text{NH}_3\text{-NH}_4\text{Cl}$  buffer to adjust the solution to ~pH 10. Add 2-3

drops of EBT indicator to the Erlenmeyer flask and titrate with EDTA. Perform this analysis in triplicate.

### Calculations

Since EDTA forms a 1:1 complex with  $\text{Ca}^{2+}$  under these experimental conditions, the millimoles of calcium present can be found by calculating the number of millimoles of EDTA consumed during the titration using the equation

$$\text{millimoles EDTA} = \text{mL EDTA} \times \text{molarity EDTA}$$

Since the total volume of the 250 mL volumetric flask has not been titrated, the millimoles of calcium titrated (calculated from the above equation) are the millimoles present in the aliquot titrated. A dilution factor must be used to calculate the total number of millimoles of metal present in the 250.00 mL volumetric flask:

$$\text{Total millimoles calcium} = \text{millimoles calcium} \times (250.00 \text{ mL} / 50.00 \text{ mL})$$

Once the total number of millimoles of calcium present in the sample is calculated, the mass of calcium, in milligrams, is readily determined.

Laboratory report.

A sample report is shown at the end of the procedure.

**WASTE DISPOSAL:** All solutions used in this experiment can be poured down the sink drain.

SAMPLE REPORT

Determination of Ca<sup>2+</sup>  
 Unknown #3.14159265358979323846  
 Lothar of the hill people

Standardization of EDTA solution:

Mass of calcium carbonate used: 0.5112 g

Standard Ca <sup>2+</sup> Solution:	Sample 1	Sample 2	Sample 3
Volume Ca <sup>2+</sup> , mL	25.00	25.00	25.00
Molarity Ca <sup>2+</sup>	0.01022	0.01022	0.01022
Vol. EDTA, mL	25.13	25.16	25.09
Molarity, EDTA*	0.010167	0.010155	0.010183
Mean EDTA molarity	0.01017		

$$*M_1V_2 = M_2V_2$$

Analysis of calcium unknown:

Ca <sup>2+</sup> Unknown:	Sample 1	Sample 2	Sample 3
Vol. Ca <sup>2+</sup> unknown, mL	50.00	50.00	50.00
Vol. EDTA, mL	37.96	37.99	37.91
Ca <sup>2+</sup> mg, aliquot <sup>1</sup>	15.548	15.561	15.528
Ca <sup>2+</sup> mg, unknown <sup>2</sup>	77.740	77.805	77.640
Mean Ca <sup>2+</sup> mg, unknown	77.73		

$$^1\text{mL EDTA} \times \text{Molarity EDTA} = \text{mmoles EDTA} = \text{mmoles Ca}^{+2}$$

$$\text{mmoles Ca}^{+2} \times 40.078 \text{ g/mole} = \text{mmoles Ca}^{+2}, \text{ aliquot}$$

$$^2(\text{Ca}^{+2} \text{ mg, aliquot}) \times (250.00 \text{ mL}/50.00\text{mL}) = \text{Ca}^{+2} \text{ mg, unknown}$$

Analysis of eggshell:

Mass of eggshell used: 0.1997 g

Eggshell solution:	Sample 1	Sample 2	Sample 3
Vol. titrated, mL	25.00	25.00	25.00
Vol. EDTA, mL	18.47	19.55	19.58
Ca <sup>2+</sup> mg, aliquot <sup>3</sup>	7.5653	7.5795	7.5880
Ca <sup>2+</sup> mg, solution <sup>4</sup>	75.6523	75.795	75.880
% Ca <sup>2+</sup> , eggshell <sup>5</sup>	37.883	37.954	37.997
Avg % Ca <sup>2+</sup> , eggshell	37.94		

<sup>3</sup>mL EDTA x Molarity EDTA = mmoles EDTA = mmoles Ca<sup>2+</sup>

mmoles Ca<sup>2+</sup> x 40.078 g/mole = mmoles Ca<sup>2+</sup>, aliquot

<sup>4</sup>(Ca<sup>2+</sup> mg, aliquot) x (250.00 mL/25.00mL) = Ca<sup>2+</sup> mg, unknown

<sup>5</sup>(mg Ca<sup>2+</sup>/mg eggshell) \*100