

# EDTA Complexometric Titration of Hydroxyapatite Column Effluent

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## Introduction

Ceramic Hydroxyapatite (CHT™) is a mixed-mode chromatographic resin widely used for the purification of proteins and monoclonal antibodies. CHT is a sintered form of hydroxyapatite ( $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ ), which is a form of calcium phosphate. The solubility of CHT increases at an acidic pH ( $\text{pH} < 7.0$ ) and it dissolves into its constituents ions ( $\text{Ca}^{2+}$ ,  $\text{PO}_4^{3-}$ ,  $\text{OH}^-$ ). Thus, the solubility of CHT can be estimated by measuring the concentration of the calcium ion, the phosphate ion, or the hydroxyl anion. Of these three ions, calcium can be quantified in a relatively simple and selective manner by complexometric titration (Belcher et al. 1958, Kim and Vipulanandan 2003). The protocol described herein presents the application of EDTA complexometric titration to quantify the total calcium concentration present in the effluent of a given CHT packed bed. The protocol has been modified such that it can be applied to either low-buffering or high-buffering capacity samples, both typically encountered during purification protocols using CHT.

## Section 1: Protocol

### Materials and Methods

#### Chemicals

Eriochrome black T (EBT), triethanolamine (TEA), ammonium chloride ( $\text{NH}_4\text{Cl}$ ), magnesium sulfate heptahydrate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ), ammonium hydroxide solution ( $\text{NH}_4\text{OH}$ ), disodium dihydrogen ethylenediaminetetraacetate ( $\text{Na}_2\text{EDTA}$ ), 10 N sodium hydroxide ( $\text{NaOH}$ ) solution, 1 N hydrochloric acid ( $\text{HCl}$ ) solution, and deionized (DI) water ( $18 \Omega/\text{cm}$ )

#### Titration Solutions

- Ammonia buffering solution

The ammonia buffering solution is made by mixing two solutions (solution A and solution B). Solution A is prepared by dissolving 1.179 g of  $\text{Na}_2\text{EDTA}$  and 750 mg of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in a final volume of 50 ml of DI water. Solution B is prepared by dissolving 16.9 g of  $\text{NH}_4\text{Cl}$  in 143 ml of ammonium hydroxide solution. The ammonia buffering solution (solution C) is prepared by mixing solution A and solution B in a 250 ml glass volumetric flask and filling the container to 250 ml with DI water.

- 0.01 M EDTA (titrant) solution

Dissolve 3.723 g of  $\text{Na}_2\text{EDTA}$  in DI water to a final volume of 1000 ml.

- EBT (indicator) solution

An EBT stock solution is prepared by dissolving 1 g of EBT in 100 g of TEA. The EBT stock solution is diluted 5x with DI water to make the working EBT solution (WEBT).

### Experimental Procedure

#### 1. Sample pH Adjustment

The pH of the sample should be adjusted to  $10 \pm 0.1$  before the start of the titration. Depending on the buffering capacity of the sample to be analyzed, the pH may be adjusted as follows:

- Samples with low total buffering capacity ( $\leq 10$  mM total buffering species):  
The pH of these samples may be adjusted to  $10 \pm 0.1$  with the ammonia buffering solution alone.
- Samples with relatively high buffering capacity ( $> 10$  mM total buffering species):  
To adjust the pH of these samples it is recommended to use a 10 N  $\text{NaOH}$  solution to neutralize the buffering species. Then add ammonia buffering solution to adjust the sample pH to  $10 \pm 0.1$ .

#### 2. Adding the WEBT Solution

Once the sample pH is within  $10 \pm 0.1$  proceed to add the working indicator solution (WEBT solution). Typically the amount of WEBT needed will be 2–3  $\mu\text{l}$  per ml of sample.

#### 3. Titration

Once the WEBT is mixed and a pink color develops titration can begin. Prior to titration, record the current EDTA titrant volume ( $V_1$ ). Begin titrant addition while monitoring the color of the sample. The titration will come to an end once the color of the sample changes from pink to purple to dark blue and finally to sky blue (end point).

End point reversibility, which is typically observed at calcium concentrations  $\geq 5$  ppm, may occur. Thus, it is recommended to wait at least 2 minutes after reaching the titration end point before reading the final volume of titrant ( $V_2$ ).

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#### 4. Calculations

- For a sample volume of 100 ml:

$$C_{\text{Calcium}} = \frac{1 \text{ ppm}}{0.25 \text{ ml titrant}} (V_2 - V_1)$$

- For a sample volume of 40 ml:

$$C_{\text{Calcium}} = \frac{1 \text{ ppm}}{0.10 \text{ ml titrant}} (V_2 - V_1)$$

Where  $V_1$  and  $V_2$  represent the volume of titrant in ml before and after titration, respectively.  $C_{\text{Calcium}}$  represents the total calcium concentration (ppm).

#### Equipment and Supplies

Magnetic stir plate, precision pipets, pH meter, 25 ml glass burette, 150 ml glass beaker, 250 ml glass volumetric flask, and PTFE-coated stir bar.

### Section 2: Tips for Better Results

#### Sample pH Adjustment

##### Gels and Membranes

- The volume of 10 N NaOH required typically varies between 0.1–1 ml
- After the NaOH step, it is recommended to use at least 0.1 ml of the ammonia buffering solution to adjust the final pH to  $10 \pm 0.1$
- If the pH goes beyond  $10 \pm 0.1$  during NaOH addition, use 1 N HCl to bring the pH back down such that the volume of ammonia buffer needed to bring the pH finally to  $10 \pm 0.1$  is no less than 0.1 ml

##### Adding the WEBT Solution

- A pink color should develop upon addition of the EBT solution. The intensity of the color depends strongly on the calcium concentration present, the EBT volume added, and the volume of ammonia buffer added

##### Titration

- For a sample volume of 100 ml, 0.25 ml of EDTA solution titrates 1 ppm of calcium
- A sample volume of 100 ml is recommended to increase the accuracy of the method especially at low calcium concentrations (e.g. <1 ppm)

#### References

- Belcher R et al. (1958). The complexometric titration of calcium in the presence of magnesium a critical study. *Talanta* 1, 238–244.
- Kim J and Vipulanandan C (2003). Effect of pH, sulfate and sodium on the EDTA titration of calcium. *Cem Concr Res* 33, 621–627.



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