High-Throughput Calcium Analysis of Hydroxyapatite Column Effluent

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Introduction

Ceramic hydroxyapatite (CHT) is a mixed-mode chromatographic media widely used for the purification of proteins and monoclonal antibodies. CHT is a sintered form of hydroxyapatite ($Ca_5(PO_4)_3OH$), which is a form of calcium phosphate. The solubility of CHT increases at an acidic pH (pH <6.5) and it dissolves into its constituent ions (Ca^{2+}, PO_4^{3-}, OH^-). Thus, the solubility of CHT can be estimated by measuring the concentration of the calcium ion, the phosphate ion, or the hydroxyl anion. Adding calcium and phosphate to buffers significantly enhances CHT lifetime under process conditions. The protocol described herein provides a rapid high-throughput method 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 or high buffering capacity samples, both typically encountered during purification protocols using CHT.

Materials and Methods

Chemicals

Calcium chloride dihydrate ($CaCl_2{\cdot}2H_20$), deionized (DI) water (18 MΩ/cm), and the QuantiChrom calcium assay kit from BioAssay Systems (www.bioassaysys.com) are required.

Note: Chelating substances will interfere with the QuantiChrom calcium assay kit.

Equipment and Supplies

Precision pipets, flat bottom 96-well polystyrene plates, and a microtiter plate reader are required.

Preparation of Working Reagent

The working reagent (WR) is prepared by mixing equal volumes of reagents A and B, both provided in the QuantiChrom calcium assay kit.

Preparation of Calcium Standards and Construction of the Calibration Line

A calcium stock solution of 200 ppm is provided with the QuantiChrom calcium assay kit. Depending on the sample volume to be used, prepare a dilution series with DI water that covers the linear range. For 25 µl of sample, the linear range will be between 0 and 40 ppm. For 50 µl of sample, the linear range will be between 0 and 20 ppm. Use DI water as the blank. Analyze the calcium standards according to section 1.4 in the kit instructions. For each sample, determine the optical density (OD) at 612 nm and correct it by the OD at 612 nm of the blank. Then plot the corrected OD at 612 nm vs. the known calcium concentration, fit the trend to a linear model, and determine the slope (m) of the line.

Experimental Procedure

96-Well Plate Calcium Analysis

The analysis is performed using the following procedure:

1. Dispense a predetermined sample (unknown, standard, blank) volume into a selected well. Repeat this step until all samples are in the plate. Up to 48 samples in duplicate can be analyzed at one time using this protocol.

Note: A sample volume between 25 and 50 µl is recommended to increase the accuracy of the assay. The linearity of the calibration changes with the sample volume used. For 25 µl of sample, the linear range will be between 0 and 40 ppm. For 50 µl, the linear range will be between 0 and 20 ppm.

2. Using a multichannel pipet, add 200 µl of WR to all the wells containing samples. Mix the samples and WR in the well by aspirating and redispensing the samples at least four times. Eliminate any air bubbles that might have been introduced by lightly tapping the plate.

3. Incubate the samples at room temperature for 3 min. The start of the incubation time corresponds to the moment WR is added to the samples.

4. Read the OD at 612 nm.

5. The calcium concentration of the unknown samples is calculated as follows:

$$Ca^{2+}\ \text{total} = \frac{OD_{Sample} - OD_{Blank}}{m}$$

where OD_{Sample} and OD_{Blank} represent the OD at 612 nm of the sample and blank, respectively, and m represents the slope of the calibration line.

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