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Understanding and mitigating conductivity transitions in weak cation exchange chromatography

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ABSTRACT

Large conductivity fluctuations were observed during a high pH wash step in a weak cation exchange chromatography process. These conductivity transitions resulted in a conductivity drop during pH increase and a conductivity rise during pH decrease. In some cases, the absolute conductivity change was greater than 6 mS/cm which was sufficient to affect target protein retention on the column. Further investigation revealed that wash buffer concentration, resin ligand density, and resin ligand pK have a profound effect on the magnitude of the conductivity transitions and the shape of corresponding pH traces. A potentiometric electrode selective for sodium ions was used to measure effluent counterion concentrations from two preparative resins during high pH washes, and the number of exchangeable counterions was compared to predictions made using ion exchange equilibrium theory. Results from this analysis show that conductivity transitions can be effectively mitigated without compromising process performance by optimizing the trade-off between wash buffer concentration and wash phase duration.

1. Introduction

Weak cation exchange resins have been widely employed for the chromatographic capture and polishing of both naturally occurring and recombinant proteins [1–9]. These resins are typically functionalized with carboxylic acid (COOH) groups that are deprotonated at intermediate and high pH. These functional groups are often referred to as carboxymethyl, or "CM", groups when the acid moiety is attached to the resin base matrix through a linker containing a methylene group adjacent to the carboxylic acid group. This is in contrast to so-called strong cation exchange resins which generally carry sulfonate (SO_3^-), or "S", functional groups that are protonated only at very low pH. The classification of CM and S resins as "weak" and "strong", respectively, follows from the fact that S groups are deprotonated over a wider pH range than CM groups. Protein retention on strong cation exchange resins is usually greater than on weak cation exchangers with identical base matrices [10]. However, there have been instances where the opposite trend was observed [11,12]. Further, some researchers have demonstrated that hydrophobic interactions play a significant role in retention on cation exchange, and that the hydrophobic contribution to retention on equivalent weak and strong cation exchange resins can be very different at high salt concentration [13,14]. In some separation processes, a CM resin may provide better selectivity because the difference in retention of the target protein and contaminant proteins is maximized. If the CM resin employed also provides high ligand density, then the sorbent may provide enhanced binding capacity and salt-tolerance along with favorable selectivity. One of the CM resins employed in this study, CM Ceramic HyperD, was evaluated with these considerations in mind.

However, it has been reported that the use of weak cation exchange resins can be complicated by the fact that transient changes in pH may accompany sudden shifts in salt concentration such as those that are used to elute target proteins [15–18]. For instance, an unwanted, temporary pH decrease was observed during a high salt protein elution step even though both the high and low salt mobile phases were buffered at the same pH. This pH transition was attributed to the exchange of protons for positively charged buffer counterions on the resin during the salt increase [16]. Since bind-and-elute cation exchange chromatography steps are routinely operated near the pK of carboxylic acid groups, these issues are anticipated to be more pronounced when working with weak cation exchange resins.

Here, we observed that significant conductivity transitions may result from step changes in pH on weak cation exchange resins. This occurred during development of a high pH wash intended for selective removal of host cell proteins prior to elution of a recombinant monoclonal antibody. The initial increase in pH was accompanied by an unexpected, temporary decrease in conductivity, and the subsequent decrease in pH was accompanied by a temporary increase in conductivity. This occurred even when the counterion (sodium) concentrations of the low pH and high pH wash buffers

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were matched. Blank runs in the absence of protein confirmed that this effect was indeed related to the behavior of the resin and was not the result of protein elution at elevated pH. Further, this effect was not limited only to a particular resin.

It has been shown that conductivity transitions can result from step increases in pH on a strong S-type cation exchange resin that also contains weak acid CM groups [19]. This result was consistent with a local equilibrium model that predicted a decrease in counterion concentration at the outlet of the column. However, this analysis did not include a direct comparison of experimental and theoretical counterion profiles.

In this study, we investigated the effects of resin properties such as ligand density and pK and process conditions such as wash buffer concentration on the magnitude of conductivity transitions during high pH washes. We used a potentiometric electrode selective for sodium ions in an effort to measure effluent counterion concentrations directly and determine the total number of exchangeable counterions during high pH washes. As anticipated, high ligand density led to more exchange of counterions and conductivity transitions of greater magnitude. Thus, the practical benefits provided by high ligand density may require adoption of strategies to mitigate conductivity transitions. An understanding of these relationships and their effect on conductivity transitions could potentially save process developers a great deal of time on empirical optimization experiments.

An additional objective of this work was to determine whether the observed conductivity transitions could be mitigated or eliminated by using a pH gradient in place of the step change. In this case, we tested the efficacy of a relatively short gradient that would be amenable to implementation in a large-scale purification process.

1.1. Ion exchange equilibrium theory

The number of counterions changing phase upon a pH shift (N_{Na^+}) can be calculated from the difference in the concentration of deprotonated CM groups in the adsorbed phase (q_{Na^+}) at high pH and low pH:

$$N_{Na^+} = \left(q_{Na^+}^{\text{high pH}} - q_{Na^+}^{\text{low pH}}\right) \cdot V \tag{1}$$

Here the adsorbed phase concentrations are in units of moles per liter of packed bed and V is the bed volume. In this case, N_{Na^+} is expressed in moles. The number of counterions desorbing from the resin upon pH decrease should be equal to the number of counterions adsorbing to the resin upon a pH increase for the equal but opposite change in pH.

q_{Na^+} is related to the dissociation constant of the CM group (K), the total concentration of CM ligands on the resin (q_R), the solution phase concentration of sodium ions (C_{Na^+}), and the solution phase concentration of H^+ ions (C_{H^+}) [17,20]:

$$K = \frac{{q_{Na^+}}^2 C_{H^+}}{(q_R - q_{Na^+})\, C_{Na^+}} \tag{2}$$

which can be re-arranged to:

$${q_{Na^+}}^2 C_{H^+} + q_{Na^+} K C_{Na^+} - K q_R C_{Na^+} = 0 \tag{3}$$

Application of the quadratic formula yields:

$$q_{Na^+} = \frac{1}{2}\left[\frac{-KC_{Na^+} + \sqrt{(KC_{Na^+})^2 + 4KC_{Na^+}C_{H^+}q_R}}{C_{H^+}}\right] \tag{4}$$

For a chromatography column at equilibrium, C_{H+} and C_{Na^+} are calculated directly from the pH and counterion concentration of the mobile phase buffer. Thus, K and q_R are the only constants needed to perform a balance on counterions from one steady-state set of buffer conditions to another.

K and q_R can be estimated by fitting a theoretical resin titration curve based on Eq. (4) to an experimental resin titration curve. In this process, an experimental value of q_{Na^+} is calculated from a mass balance on sodium ions in the resin slurry being titrated:

$$q_{Na^+} = \frac{m_{Na^+} - C_{Na^+}(V_o + (m_{Na^+}/N)) + C_{Na^+}^{\text{init}} V_o}{V} \tag{5}$$

Here, m_{Na^+} is the moles of NaOH titrant added to the resin slurry, N is the normality of the titrant, V_o is the initial volume of resin slurry, and $C_{Na^+}^{\text{init}}$ is the initial concentration of sodium chloride in the resin slurry. The value of C_{Na^+} is calculated from the solution phase electroneutrality condition:

$$C_{Na^+} + C_{H^+} = C_{Cl^-} + C_{OH^-} \tag{6}$$

Eq. (6) can be re-arranged to solve explicitly for C_{Na^+}:

$$C_{Na^+} = C_{Cl^-} + \frac{K_W}{10^{-\text{pH}}} - 10^{-\text{pH}} \tag{7}$$

where K_W is the dissociation constant of water and C_{Cl^-} is equal to $C_{Na^+}^{\text{init}}$ after adjusting for volume change due to titrant addition. Best-fit values of K and q_R can then be regressed through sum of least-squares analysis. In this study, the difference in theoretical (Eq. (4)) and experimental (Eq. (5)) values of q_{Na^+} across the entire titration curve was minimized.

2. Materials and methods

2.1. Resins and columns

Experiments were performed on CM Ceramic HyperD, S Ceramic HyperD (Pall Corporation, East Hills, NY) and CM Sepharose FF (GE Healthcare, Piscataway, NJ) cation exchange resins. For column chromatography experiments, the resins were packed into 0.66 cm i.d. × 20 cm Omnifit columns (Bio-Chem Valve, Inc., Cambridge, England).

2.2. Dynamic binding capacity measurements

The resin dynamic binding capacities at 1% breakthrough ($DBC_{1\%}$) were measured using a full-length monoclonal antibody. In this case, the resins were evaluated for direct antibody capture from harvested cell culture fluid. Measurements were made using an AKTA Explorer 100 FPLC (GE Healthcare, Piscataway, NJ). For purposes of this study, 1% breakthrough was defined as the column load density (grams of antibody loaded per liter of packed bed) at which antibody concentration in the column effluent was equal to one percent of the antibody concentration in the load.

Flow rates were selected based on manufacturer recommendations and known pressure limitations at manufacturing scale. CM Ceramic HyperD was loaded at a linear velocity of 600 cm/h, and CM Sepharose FF was loaded at 200 cm/h.

Full-length monoclonal antibodies were expressed in Chinese hamster ovary (CHO) cells in 250 or 400 L bioreactors at Genentech (South San Francisco, CA). Cell culture fluid was harvested using depth filtration and sterile filtration. Harvested cell culture fluid (HCCF) was adjusted to pH 5.5 by adding 1.0 M acetic acid. HCCF conductivity was adjusted to 6 mS/cm by adding purified water. Conditioned HCCF was sterile filtered prior to column loading.

Column effluent was collected in 15 mL fractions, and the concentration of antibody in the fractions was quantified with an HPLC assay using a POROS Protein A (2.1 mm × 30 mm) affinity column (cat. No. 1-5024-12) from Applied Biosystems (Foster City, CA). The column was run at 2.0 mL/min at ambient temperature for a duration of 6 min. The column was equilibrated with phosphate-buffered saline (PBS) at pH 7.2. Samples were injected without dilution and antibody was eluted with PBS adjusted to pH 2.0 (with