

Theory and Introduction: Ion exchange chromatography involves two primary steps, first the binding of a protein to a charged resin and second the elution or displacement of the protein from the charges of the resin. Critical to the former are the pH of the buffer used to equilibrate and load the proteins onto the column. Factors that control the elution are pH or ionic strength. Common ion exchangers include the positively charged anion exchanger - DEAE (diethylaminoethyl) and the negatively charged cation exchanger - CM (carboxymethyl). Review the references in the purification handout for good detailed references.

Important Points to Consider For Ion Exchange Chromatography

- **Preparation of resin** - Before each use, the resin should be prepared and regenerated to remove old proteins and ensure that the proper counterion (a counterion is the ion bound to the charged resin) is in place. To achieve this, simply wash the column with a buffer containing 1M of the salt of preference. NaCl or KCl are typically used. In some cases, the resin is supplied as a dry powder. Read the manufacturer's recommendation to swell the gel. Generally, simply add an equilibration buffer and mix. Let the mixture sit for one hour and pour off the buffer with the small particles "fines". Add back enough resin to more than cover the resin and swirl occasionally while incubating at room temperature overnight. You do not need to do this if your resin is already hydrated.
- **pH of buffers** - If you know or can calculate the pI of the protein. The webpage has a couple of links to good calculators. You can find the DNA and amino acid sequence of MGH on the web for your calculations. To ensure the protein will be positively charged, use at one pH unit above your protein's pI. Conversely, using a buffer whose pH is one pH unit below the pI will ensure the protein is negatively charged. The further away the pH is from the pI the more likely the proteins will bind. Remember that most proteins are unstable at extreme pH ranges 1-6 and 9-14. It is also sometimes just as good to have a protein not bind and allow a contaminant bind to remove stubborn proteins from your purification.
- **Buffer selection** - The buffer you use **MUST** be appropriate for the pH and resin you are using. Equilibration Buffer - A 10 mM buffer (phosphate (pKa = 7.2 or Tris-Cl pKa = 8.06) will be the right concentration. Review your lab math for how to make a buffer. **REMEMBER** - all solutions must contain a buffer component plus other compounds.
- **Elution** - Use either a step/isocratic gradient to elute your protein or a gradient elution.
 - Step / Isocratic Elution- Most proteins will have been eluted by a buffer containing 500 mM NaCl. So you may want to use two or three different buffers which have salt concentrations ranging from 50 mM NaCl to 500 mM. A recommended volume for step washes/elution = 3 column volumes. Collect fractions throughout the column run.
 - Gradient Elution - There are two gradient makers in the laboratory. We are making several more. See the Tegrity file for how to use this. A good rule of thumb is to use 10 times the column volume for your gradient. That means for a 10 ml column, a 100 ml gradient will work well. To prepare this kind of gradient, 50 ml of the buffer without salt is placed in the container that will plumb to the column and 50 ml of buffer with the final salt concentration is placed into the other container. A gradient concentration of 0 to 250-500 mM NaCl in the buffer will work well. Don't forget to wash with at least two column volumes of 1 M NaCl at the end of the gradient to remove other proteins (or MGH if it hasn't already eluted).

General Protocol for MGH Purification Using DEAE or CM -

1. Prepare a 10 ml column using the glass column, attach the pump and adaptor and use a 1-2 ml/min flow rate.
2. Regenerate with a 25 ml wash High Salt Buffer (10 mM buffer at your pH plus 1M NaCl).
3. Equilibrate the column with 50 - 100 ml of Equilibration Buffer (10 mM buffer at your pH)
4. Check to see if you sample has more than 50 mM NaCl or KCl. If it does, it must be dialyzed overnight before using. Alternatively, you can dilute the sample with equilibria buffer until the salt concentration is equal to or less than 50 mM. If starting from lysates, no further preparation of the sample is necessary.
5. Save a sample of the lysates for later analysis. Freeze in a microfuge tube.
6. Load the column with your sample. 1-2 ml / min flow rate.
7. Wash with 3 column volumes of Equilibration Buffer.
8. Elute protein using either a isocratic (40 ml) or gradient elution (100 ml total). Collect 2-5 ml fractions throughout this step.
9. Follow up with a High Salt buffer wash to remove any tightly bound proteins and regenerate your resin for the next use. Collect 2-5 ml fractions throughout this step.
10. Analyze each tube for the protein for total protein concentration (Bradford assay) and MGH (Fluorescence Assay).
11. Prepare a chromatograph showing both total protein concentration and MGH concentration for the samples.
12. Pool fractions as indicated in the purification handout.