

Theory and Introduction: Size exclusion chromatography (SEC) is also often called molecular sieve, gel permeation or gel filtration chromatography. This analytical method separates molecules based on their molecular sizes and shapes by using the molecular sieve properties of a variety of porous materials. Gel filtration is a powerful and popular method to purify and determine the molecular weight of proteins. The SEC column is packed with a resin consisting of beads with pores of defined size. This resin is the SEC stationary phase. The mobile phase is a buffer that usually contains at least 100 mM salt. The salt is necessary to reduce the interaction between the proteins and the beads. The size of the pores or "holes" in these beads is controlled during the manufacturing process. The separation of chemicals chromatographed by SEC is based on the amount of time molecules spend included in the pores of these beads. SEC is used to separate and analyze a variety of chemicals, including both synthetic polymers and biopolymers. It is a powerful and popular method to purify and determine the molecular weight of proteins.

The total volume of a SEC column may be regarded as containing three compartments, V_o , V_i and V_g . The void volume V_o represents the volume outside of the beads. V_o is determined by using a very large molecule that is larger than the exclusion range for the gel. Blue Dextran is most often used for this purpose. Blue dextran is a very large polysaccharide with a blue dye covalently bonded which has a molecular weight of about 2,000,000 Da. The space within the beads is referred to as the inclusion or internal volume, V_i . To determine this quantity, a very small molecule is used. Typically, an amino acid linked to a fluorescent molecule such as dinotrophenyl or ascorbic acid is used to find V_i . These molecules all have molecular weights much lower than the low end of the fractionation range of most SEC gels and therefore are "included" in the pores of the beads. The actual volume that it requires to "elute" a biomolecule from a SEC column is called the elution volume, V_e . For a given SEC column to be useful for separating molecules, V_e should fall between the V_o and the V_i . Review the references in the purification handout for good detailed references.

Important Points to Consider For Size Exclusion Chromatography

- Preparation of resin - If using new resin that has not been "de-fined" (usually when starting from powder) it is important that the small particles are removed before using. To do this, hydrate the resin in buffer for about one hour. Pour off the buffer and the particles that have not settled yet. You may need to resuspend and repeat several times. This is not necessary for most of the resins we are using in this laboratory.
- Column and Sample Preparation - The ratio of the length vs the diameter of the column should be 20 to 100. In our experiments, using the taller glass columns (located at the front of the room - pre, poured). The sample must be concentrated to a small volume for effective resolution. The volume of matrix should be 30 to 100 times the sample load volume. Using a large, column or concentrating the protein load by ammonium sulfate precipitation or ultra filtration achieves this.
- Buffer selection - The salt concentration is not critical as the protein does not bind to the resin, but instead goes around or through the beads. However, it is good practice to include a 100 to 200 mM salt in the buffer. This helps to reduce non-specific interactions between the protein and the resin. REMEMBER - all solutions must contain a buffer component plus other compounds.
- Elution - Prepare the column with at least three column volumes of your equilibration buffer before loading the sample. Maintain a constant flow rate throughout the column run. A flow rate of 1-1.5 ml/min will suffice.

General Protocol for MGH Purification Using SEC -

1. Prepare a tall glass columns with the blue plastic, attach the pump and adaptor and use a 1-1.5 ml/min flow rate.
2. Equilibrate the column with 100 ml of Equilibration Buffer (10 mM buffer at your pH plus salt - see above for concentration)
3. Save a sample of the lysates for later analysis. Freeze in a microfuge tube.
4. Load the column with your sample. 1-1.5 ml / min flow rate.
5. Wash with 20 ml of Equilibration Buffer and start to collect 2 ml fractions.
6. Continue to collect fractions until the MGH has run through the column. Continue to collect for at least another 10 ml.
7. Analyze each tube for the protein for total protein concentration (Bradford assay) and MGH (Fluorescence Assay).
8. Prepare a chromatograph showing both total protein concentration and MGH concentration for the samples.
9. Pool fractions as indicated in the purification handout.

Prepare 100 ml of the SEC Equilibration buffer.