Journal Format Lab Reports

Journal format lab reports must be type-written (with at least 1.5 line spacing) and graphs should be done using a spreadsheet program that will allow you to insert trendlines and derive equations for trendlines as necessary. When writing the reports, remember that you are writing for an intelligent reader who is familiar with biochemistry and the techniques used in typical lab experiments, but who has not performed the actual experiment on which you are reporting.

I. Title

II. Abstract – one paragraph of no more than ~300 words. The abstract should be a stand-alone summary of the report. It should include what has been accomplished and the significance of the work.
   a. Statement(s) of the question(s) being answered.
   b. 1-2 sentences on the system being studied (if relevant).
   c. Statements of the techniques being used to carry out the experiment and assay the results.
   d. Statement(s) of the specific results obtained (e.g. Hemoglobin and chromate were successfully chromatographically separated and recovered from the column in 87% and 65% yield, respectively). All quantitative results should appear with the corresponding units and should be rounded to the appropriate number of significant figures.

III. Introduction – This section should be written in the present tense and should include a summary of the objectives of the experiment, a justification for doing the experiment and a summary of the approach taken. The intro must touch on background information from all major relevant areas bearing on the experiment. "Relevant areas" will include background on relevant aspects of the enzyme on which the experiment being run, background on any separation or purification techniques being implemented to prepare the sample and any assay techniques used to quantitate results. Cite references whenever appropriate.

IV. Materials and Methods – This section should be written in the past tense and must not be copied verbatim from the lab manual nor duplicated from another student's lab report. Do not make a list of all reagents and equipment and do not "reproduce" the tables of the lab manual. Outline in sentence form the procedures you used, together with reagents and equipment, so that an intelligent biochemist could reconstruct the details by thinking about what you wrote. Do not give details about volumes of each component, instead give the final volume for the reaction along with the final concentrations of each of the components. It should be sufficiently detailed that someone else would be able to reproduce the results. Use subtitles to separate each method described. Do not put any results in this section.

V. Results – This section should present (in text format) what you have found and what it means. It should be written in the past tense; report only your findings from the collected data. Readers are most interested in what you found out, not the gory details of how you found it out (which is the purpose of the Materials and Methods section and the Appendix). Make sure you write this in prose even though, to an extent, you are usually going to be going down a "list" of things in the lab manual. It is fine if answers are short and to-the-point, but they MUST be complete sentences.

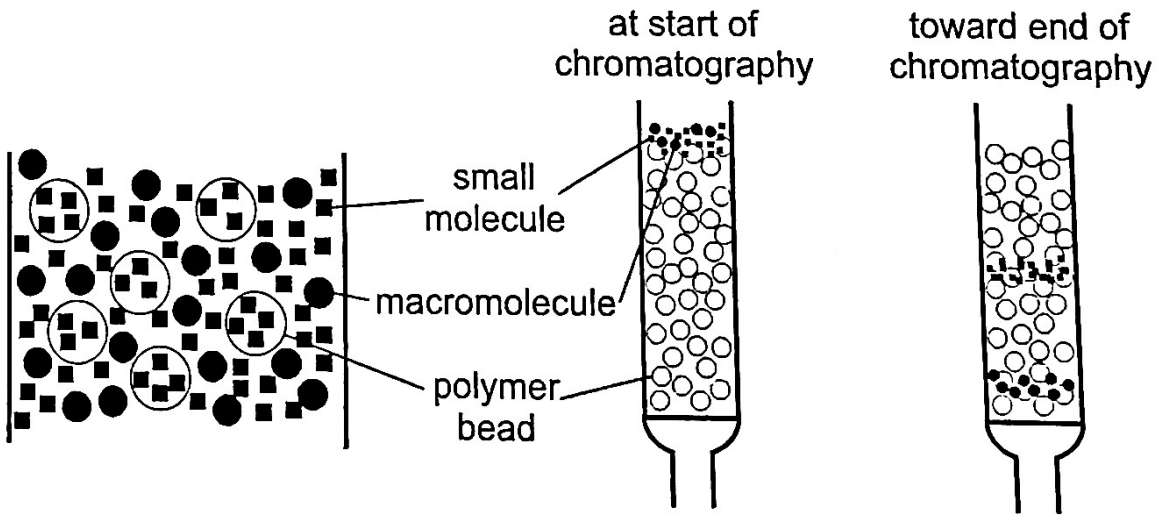
Gel Filtration Chromatography

Introduction

Many different types of molecules may be separated from one another strictly on the basis of molecular size through the use of materials often called "molecular sieves." Actually, these are inverse sieves, in that the larger molecules pass through a packed column of the material more rapidly than the smaller ones.

The materials most commonly used for separating and purifying proteins are beads of polysaccharide material prepared by careful crosslinking of long polymers of glucose units (dextrans). When put in water, or better in salt solution, the particles swell and form a gel-like material. Each of the resulting gel particles has a structure similar to that of a wet sponge, with many openings leading into the interior of the particle. The size of these holes can be controlled in manufacture, and a range of materials are available with different sized openings. The choice of size depends largely on the sizes of the molecules to be separated.

In practice, a column is packed with the gel beads and the solution of molecules to be separated is added to the top, then washed through the column with water or some buffer solution. Under these circumstances, part of the solution will move through the gel particles and part will move through the small spaces between them. However, any molecules which are too large to enter the gel particle will have to remain in the solution which goes around the particles. Since this part of the solution moves down the column much more rapidly than does the portion moving through the gel particles, these large molecules will reach the bottom of the column rapidly. We say that these molecules have been "excluded" from the gel particles, and the size of the holes in a given type of gel is usually expressed in terms of the "exclusion limit" of the gel (i.e., the molecular weight of molecules that are just too large to get into the gel). For example, Sephadex G-25 excludes globular proteins having molecular weights above 5,000 and G-50 those above 30,000 Da.



The molecules that are capable of entering the gel particles move down the column much more slowly than the excluded ones. However, even among these molecules there will be differences in the rate of movement due to their relative sizes. The larger ones will more often miss the openings and bounce off the gel rather than penetrating. Thus, they will have a greater tendency to be washed on past the particle than will the smaller molecules which pass more readily through the "holes." This experiment is not designed to show this type of

separation, but it is a technique much employed for the separation of one type of protein molecule from another.

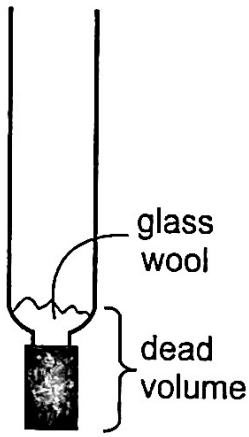
In this experiment, we will carry out the separation of a large protein molecule (hemoglobin, Hb) from a small molecule (chromate, CrO_4^{2-}), estimate gel column parameters, and determine percent recoveries.

Experimental Procedure

A. Obtain a column, outlet tube, tube clamp and 200 mL of 0.0500 M phosphate buffer, pH 7.50.

B. Estimate "dead volume" of column:

1. Put outlet tube with clamp (leave open) on end of column and mount column perpendicular to laboratory bench.
2. Place glass wool plug in column and tamp down with a glass rod so top of plug is at approximately the 9.50 mL mark.
3. Add 5.00 mL of buffer to the column and collect the buffer that comes through the column in a 10 mL graduated cylinder. The volume of buffer that remains on the column is a good estimate of its "dead volume."



C. Preparation of gel filtration column:

1. Add 10.0 mL of Sephadex G-25 suspension to your column all at once.
2. Open clamp and drain until Sephadex has settled. Add more buffer from time to time to maintain maximum flow rate. **Do not allow column to run dry.**
3. Adjust settled Sephadex with a long Pasteur pipet to give a 4.50 - 5.00 mL gel bed, if necessary.

D. Determine column drop volume while performing step C-3.

1. Collect 50 drops from column in 10 mL graduated cylinder.
2. Determine volume collected and calculate drop volume in mL/drop.

E. Calculate total column volume:

1. Measure height of settled gel bed (h) only and inner diameter of column.
2. $\pi r^2 h$ + "dead volume" = calculated total column volume.

F. Separation of hemoglobin and chromate:

1. Label 25, 15 x 125 mm or larger test tubes.

2. Make mixture of 1.00 mL of hemoglobin stock solution and 1.00 mL of chromate stock solution.

3. Sample application and chromatography.

   a. Remove excess buffer from column until buffer is just at the top of the gel bed.

   b. Carefully add 0.100 mL of F-2 (Hb and CrO_4^{2-} mixture) to top of gel using a 1.00 mL pipet.

   c. Immediately begin to collect first column fraction.

   d. After the Hb/chromate sample has entered the gel bed (collecting 1 – 2 drops of first fraction), quickly and carefully fill column to top with buffer using a long Pasteur pipet and maintain at this level for maximum flow rate. Once the sample is applied, **DO NOT** stop column flow until you have collected **ALL** your fractions.

   e. Collect the remainder of first fraction (5 drops total) and 24 more 5 drop fractions, or collect fractions until baseline absorbance after chromate peak.

4. Absorbance measurements:

   a. Add 4.00 mL of buffer to each fraction.

   b. Zero spectrophotometer with buffer.

   c. Measure A_{380} of each fraction.

   d. **Save** diluted column fractions. Do not discard them.

   e. Plot A_{380} versus elution volume in mL (total volume eluted from column) and include it in your report.

G. Estimation of Recovery of Hb and CrO_4^{2-}:

1. Combine diluted column fractions that contain Hb, measure volume (in mL), and determine A_{380}.

2. Combine diluted column fractions that contain chromate, measure volume (in mL), and determine A_{380}.

3. Prepare a solution of 0.100 mL of Hb stock solution and 20.0 mL buffer. Determine the A_{380} of this solution. Use a 100 µL automatic pipet to transfer the Hb stock solution.

4. Prepare a solution of 0.100 mL of chromate stock solution and 20.0 mL buffer. Determine the A_{380} of this solution. Use a 100 µL automatic pipet to transfer the chromate stock solution.

3. Calculate total Hb put on column in A_{380} x mL units:

$$\frac{A_{380} \text{ of step G3 solution x } 20.1 \text{ mL}}{2} = A_{380} \text{ x mL units}$$

4. Calculate total CrO_4^{2-} put on column in A_{380} x mL units:

$$\frac{A_{380} \text{ of step G4 solution x } 20.1 \text{ mL}}{2} = A_{380} \text{ x mL units}$$

7. Calculate Hb recovered from column in A_{380} x mL units:

A_{380} of pooled Hb fractions x mL pooled Hb fractions = A_{380} x mL units

8. Calculate CrO_4^{2-} recovered from column in A_{380} x mL units:

A_{380} of pooled chromate fractions x mL pooled chromate fractions = A_{380} x mL units

9. Calculate percent recovery of Hb:

$$\%\,\text{recovery} = 100 \times \frac{A_{380} \times \text{mL units of Hb eluted from column}}{A_{380} \times \text{mL units of Hb applied to column}}$$

10. Calculate percent recovery of chromate:

$$\%\,\text{recovery} = 100 \times \frac{A_{380} \times \text{mL units of chromate eluted from column}}{A_{380} \times \text{mL units of chromate applied to column}}$$