College of the Canyons
Biotechnology Program

(a) Gel bead, Gel matrix
(b) Solvent, Gel beads
(c)
(d) Small molecules, Large molecules
(e) Amount of solute / Volume of effluent
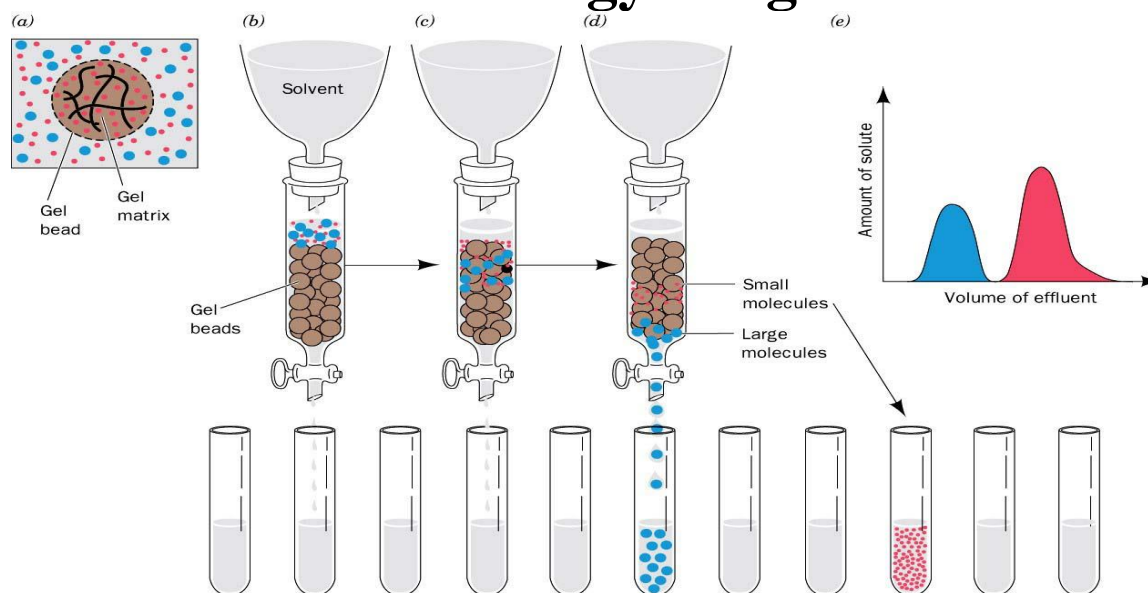
Gel Filtration: Version A

- **Gel Filtration is a chromatographic technique where by scientists use to separate one substance from another by a process known as filtration.**
- **In this experiment you will use gel filtration to separate a mixture of protein and salt. Unlike most filtration, this process will hold the small particles back and let the larger particles pass** *(sort of like making coffee backwards??)*
- **A plastic column is filled with tiny porous beads. Small molecules running through the column temporarily get trapped in the pores in the beads. Big molecules do not fit in the pores and flow between the beads and therefore exit the column before the small molecules.**
- **Purification of molecules based on size is used in the manufacture of foods and medicines as well as in scientific research and medical labs.**
- **This technique is utilized in the growing field of proteomics, which is the characterization of proteins found within cells. The mapping of the human genome was impressive, but the comprehension of proteomics correlates to reaching destinations from that map.**

Did you know that rigorous science training will make you more competitive for ANY type of job? Employers know that students who can tackle hard science will do well with almost ANY challenges presented them. For information on biotechnology and other robust science courses, contact: Jim Wolf, College of the Canyons Biotechnology Director at (661)362-3092 or email: jim.wolf@canyons.edu

GOT SCIENCE? GET AHEAD!

INTRODUCTION TO GEL FILTRATION WITH A SEPHADEX COLUMN
(SIZE-EXCLUSION CHROMATOGRAPHY, "MOLECULAR SIEVING")

I.   OBJECTIVES:

1.   To separate a protein from salt using a Sephadex column

2.   To understand the principles of gel-filtration

II.   BACKGROUND

In the first experiment, you separated the colored dyes in Kool-Aid using a C_{18} Sep-Pak. You learned that dye molecules could be separated because one dye was more polar than the other.

Another method of separating mixtures, using gel filtration, separates molecules according to their size. The chromatography column in this case is filled with microscopic beads with pores running through them. As a liquid solution containing a mixture of different sized molecules flows through the column, molecules small enough to enter the pores in the beads move slowly within beads and from bead to bead, while molecules too large to enter the pores travel more quickly between the beads. Since larger molecules are excluded from the interior of the beads, the separation is sometimes called size-exclusion chromatography. Sephadex (the commercial name for the beads used in this lab) and similar products are available with many different sized pores, so that a scientist can select a pore size just right for a particular job. Gel filtration is important because it can separate molecules gently so that they are undamaged for further study. Gel filtration is used in research , industrial testing, and medical laboratories. It plays a key role in the purification of enzymes and other proteins, polysaccharides, nucleic acids and other molecules.

III.   OVERVIEW:

In today's experiment, you will be using a prepacked Sephadex column to separate a protein from the salt solution in which it is dissolved. The protein is albumin from hen's egg, dissolved in a 0.025 molar solution of NaCl. Separating a protein from a salt is called "desalting," a common procedure. For example, a scientist may wish to purify the proteins in from the mixture we call blood. Blood contains salts similar to those in sea water, so one of the first tasks in purifying blood proteins is to free them from the salts.

The albumin molecules are thousands of times larger than the sodium and chloride ions present in solution. Both the salt and the protein are colorless, so detection will involve the use of conductivity measurements and an indicator . Since NaCl ions conduct an electrical current, you will detect relative salt quantity by measuring the electrical conductivity of each sample.   In the Bradford assay for protein, a small amount of a red indicator solution is added to each sample. If no protein is present, the solution turns brownish. If protein is present, the solution turns blue because the indicator color is changed as it binds to protein.

Predict whether the first samples to come through the Sephadex column will contain salt or protein. Explain your reasoning.

_____

GEL FILTRATION CHROMATOGRAPHY

SOLVENT FLOW

POROUS BEADS

RETARDED SMALL MOLECULE

UNRETARDED LARGE MOLECULE
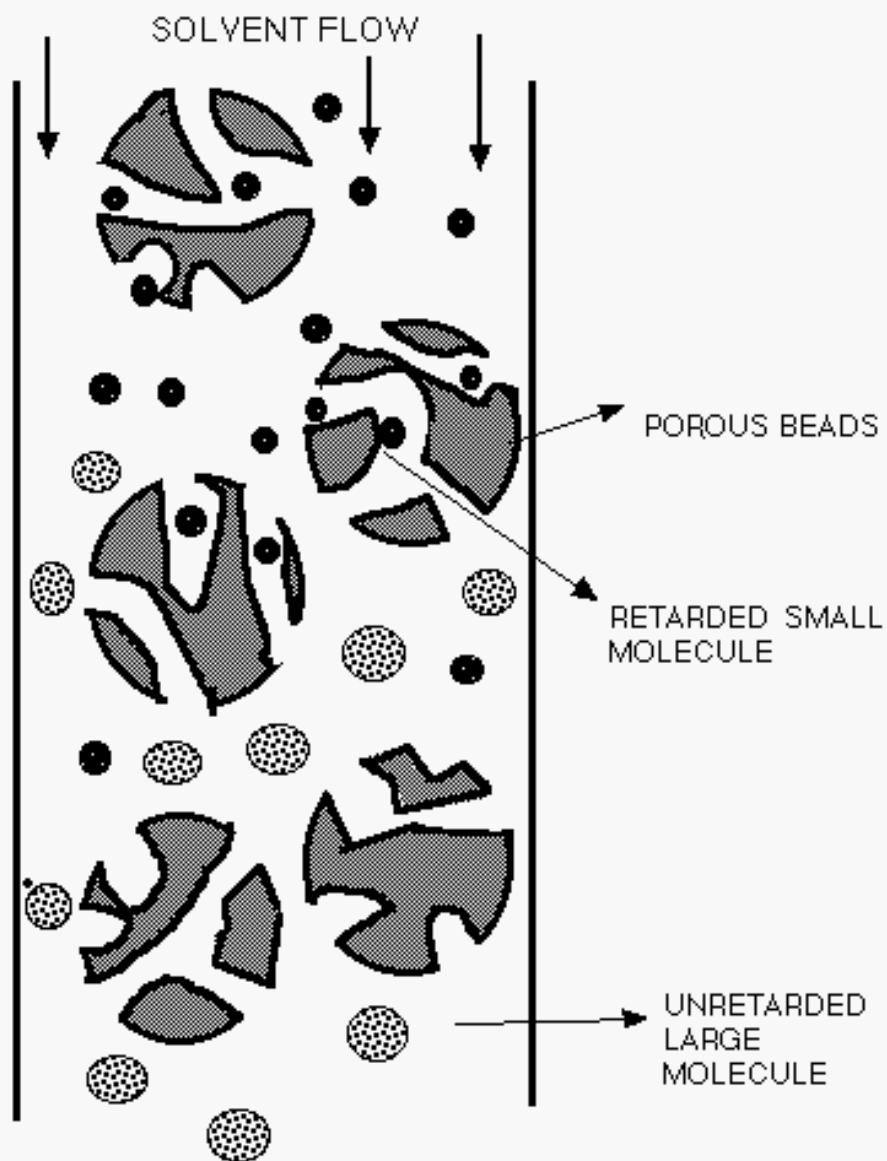
IV.   Check-List of Materials for a Lab Station
   A.   _____   11   1.5 ml test tubes
   B.   _____   marking pen
   C.   _____   test tube rack
   D.   _____   Sephadex G-25 column mounted on support.
   E.   _____   2 plastic transfer pipets
   F.   _____   albumin in NaCl: 1.8 ml in a plastic tube
   G.   _____   Bradford reagent in a plastic tube
   H.   _____   conductivity meter
   I.   _____   cup to clean electrodes
   J.   _____   squeeze bottle of deionized water
   K.   _____   cup for waste from the column
   L.   _____   Bradford color chart

V.   PROCEDURE - PRELIMINARY STEPS:
   A.   Fill the squeeze bottle with deionized water.

   B.   Remove top and bottom caps from the column, and let the deionized water left in the column during storage drain out of the column into a waste cup. Place the caps where they won't be lost.
   C.   Label the 10 test tubes 1 to 10, and line them up in the test tube rack.

   D.   Label one tube "control", and with a transfer pipette, add 19 drops (0.8 ml) of the albumin/NaCl mixture. Set this tube in the back of the test tube rack.

VI.   THE SEPARATION
   A.   When the column has drained, carefully position the test tube rack with its marked test tubes directly under the column. Test tube #1 should be ready to collect the first drops from the column.

   B.   Carefully pour the remaining albumin/NaCl mixture onto the top of the column.

   C.   Collect 19 drops (0.8 ml) in tube #l and then move on to tube #2.

   D.   Continue to collect 19 drop samples in the correct sequence in the test tubes. When the column stops dripping see step E.

   E.   By the second or third tube, the entire original sample will have entered the beads, and the column will stop dripping.   At this stage, carefully add deionized water from the squeeze bottle onto the column to a height of about one centimeter. Keep counting 19 total drops per tube. When you have collected more drops and the column has again stopped dripping, carefully add more deionized water onto the column to fill it. Once you have collected all ten 0.8 ml samples, replace the caps on the column, trapping the remaining water in the column for storage. Be sure there is at least one centimeter of deionized water stored in the column.

VII.   TEST FOR ELECTRICAL CONDUCTIVITY TO INDICATE THE PRESENCE OF NaCl.

A.   Close tube # 1, invert it 2 or 3 times to mix, then immerse the conductivity meter electrodes into the liquid. Read and record the salt concentration (mg/oz) on your data table. If the meter jumps between two values, record the average of the two.

B.   Rinse the electrodes off by squirting deionized water from the squeeze bottle over them. Let the drips fall into a waste cup. Gently shake off clinging water drops.

C.   Measure and record the salt concentration for each of the other tubes. Be careful to squirt the electrodes with deionized water between samples.

D.   Measure and record the salt concentration of the control.

VIII.   TEST FOR PROTEIN: THE BRADFORD TEST

A.   With a transfer pipet add exactly 10 drops of Bradford reagent to each sample and the control. Be careful not to contaminate the pipet by allowing it to touch the samples.

B.   Close each tube securely. Invert each sample several times to thoroughly mix the indicator with the sample. On your data table record your impression of the color, and record the approximate protein concentration for each tube, using the color chart. Use color descriptions such as dull blue, bright blue, blue gray, gray brown, and so on. If your sample is between two colors on the chart, estimate the protein concentration.

IX.   INTERPRETING YOUR DATA

Make two graphs of your data with the tube number along the X axis. Put salt concentration (mg/oz) along the Y axis of one graph and protein concentration (μg/ml) along the Y axis of the other graph.

Tube Number	Salt Concentration	Bradford Color	Protein Concentration
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
Control			

Questions:

1. Were you able to separate the protein from salt? Explain how you came to this conclusion.

2. Which of your tubes contains the most concentrated protein that is salt free? Explain how you concluded this.

3. Which of your tubes contains the most concentrated salt solution with the least amount of protein? Explain how you concluded this.

4. Is there one of your samples that you might choose to run through the column a second time? Which one and why?

5. Which tube (only one tube) proves that the original solution contained a high concentration of protein and a high concentration of salt?

6.   Exercise: A scientist used a Sephadex G-100 column to determine the molecular weight of a newly discovered protein. The G-100 Sephadex beads have a larger pore size than the G-25 beads that you have just used. In the G-100 beads the pore size is large enough so that some of the proteins can enter the beads. The scientists ran three standard proteins (A, B, and C) with known molecular weights through the column and measured the elution volume. The elution volume is the total volume of water that drips from the column after adding the sample until the most concentrated protein sample is collected. Using the data below, plot the data for the standard proteins.

	Molecular Weight	**Elution Volume**
Protein A	95,000 A.M.U.	10.0 ml
Protein B	70,000 A.M.U.	25.0 ml
Protein C	13,000 A.M.U.	70.0 ml
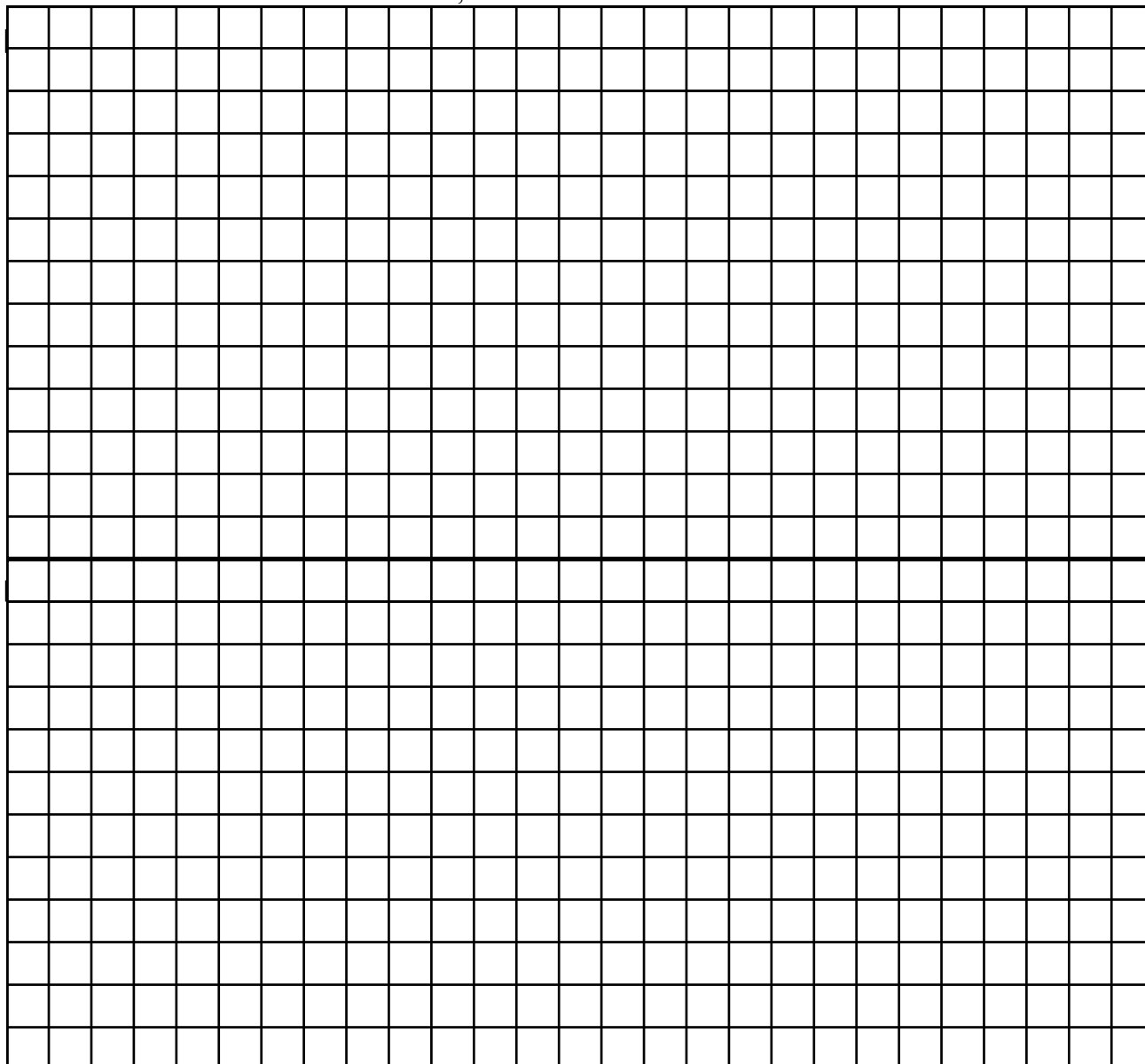
When the experimenter ran the unknown protein through the same column, it was eluted at 50 ml. Use your graph to determine its molecular weight. What is the approximate molecular weight of the unknown protein?