

Lab 10 – Size Exclusion Chromatography

See attached material for background.

Purpose:

To increase the knowledge of ways to purify proteins, practice the process of size exclusion chromatography and interpret results from a separation.

Procedure:

1. Obtain 12 collection test tubes and label ten sequentially from 1 to 10. Label the tubes with your name. Label the final two tubes "Waste" and "Column Buffer". Using a clean pipette tip, transfer 4 mL of column buffer into the tube labeled "Column Buffer."
2. Remove the top and bottom caps from sizing column. Allow all the buffer to drain into the waste tube. Observe the upper surface of the matrix and insure that all of the buffer has entered the column. Looking directly over and into the column, you should see the "grainy" appearance of the column matrix. Cap the bottom of the column.
3. Carefully place the column onto tube 1. You are now ready to load the protein sample onto the column.
4. When you are ready to load the protein mix, uncap the column. It is important to uncap the column only when you are ready to load your protein – you do not want your column to run dry. Using a micropipette add 50 μL of protein mix onto the top of the column bed. The pipette should be inserted into the column and the protein mix should be loaded **slowly** just above the top of the column so that it minimally disturbs the column bed.
5. As soon as the drop of protein mix enters the column bed, carefully add 250 μL of column buffer to the top of the column. This is best done by inserting the pipette tip into the column so that it just rests above the surface of the column matrix. Slowly let the buffer run down the side of the tube and onto the top of the bed. Begin to collect drops in tube 1.
6. Add another 250 μL of column buffer to the top of the column. Add the buffer as before, by placing the pipette tip just above the top of the column and letting the buffer run down the side of the tube. Continue to collect drops in tube 1.
7. Add 3 mL of column buffer to the top of the column matrix. This can be done by adding 1 mL three times from the micropipette. At this time the protein mix has entered the column far enough so that slight disturbances to the column bed will not affect the separation. Transfer the column to tube 2 and begin to count the drops that enter into each tube. Collect 5 drops of buffer into tube 2.
8. When 5 drops have been collected into tube 2, transfer the column onto tube 3. Collect 5 drops of buffer into each collection tube. When 5 drops have been collected into a tube, lift it off and transfer it to the next tube.
9. Continue collecting 5 drops into each tube. When you reach tube 10, collect a total of 10 drops. Cap the column and wrap parafilm around the top of each of your collection tubes.

Results:

Sketch all ten collection tubes as you observe them.

Conclusion:

1. Was the column able to separate the protein mix? Why or why not?
2. Indicate what you think is in each of your collection tubes