Name:___Justin Barry____ Date:____7/21/08_____ Period:_____ Lab Title:____Size Exclusion Chromatography_

Focus Question: Are you able to separate two types of molecules based on molecular size?

Word List:
-chromatography
-beads
-column bed
-traps
-excluded
-fractionate
-exclusion limit
-buffer
-sample
-fractions
-hemoglobin
-myoglobin
-affinity
-sickle
-carrier protein
-tubes
-Columns
-mixture

Hypothesis:
If the molecules are different molecular sizes, then you will be able to separate them using chromo graphic techniques.

Concept Map:
(attached)

Procedures:
1. Label 12 collection tubes in rack. (waste and buffer tubes)
2. Place 4 mL buffer in buffer tube.
3. Drain all buffer into waste, cap.
4. Place column onto collection tube. Load protein into column
5. Remove end cap from column. Load one drop of protein mix onto the top of the column bed.
6. Allow protein to enter column bed.
7. Add buffer to top of column- allowing drops to fall into tube #1.
8. Add more buffer to column, collecting drops.
9. Add 3 mL of buffer to column, transfer to tube #2, counting 5 drops.
10. Collect 5 drops in successive tubes and end at tube #10.
11. Cap column when finished.

Conclusion:
Because Hemoglobin is a large molecule (65,000 Daltons), while Vitamin B-12 is small, (1,350 Daltons), it can be separated using Size Exclusion techniques. As the mixture is placed in a packed column of microscopic beads, the larger molecules pass quickly around the beads, thus separating the mixture and isolating the appropriate B-12 biomolecule.

Data and Analysis:
-The protein mixture was reddish brown.
-The mixture absorbed into the column immediately after it was put in.
-After the 250 microliters were added, the protein mixture traveled down the column and eventually started to separate.
-A brown solution separated first, leaving a pink mixture at the top of the column.
-The brown solution traveled down the column extremely fast, depositing mostly in the 2nd tube.

Post Lab Questions:
1. The color was reddish-brown due to the hemoglobin (brown) and the vitamin B-12 (fuchsia).
2. The column must be dry to evenly receive the mixture. If it is not, the buffer will dilute your initial mixture.
3. You needed to add more buffer to properly embed the mixture in the column so that it would not 'wash away' after 3 ml of buffer was added. The addition of the buffer also moved the mixture down the column.
4. The greatest hemoglobin peak was in the 2nd column and the greatest Vitamin B-12 peak was in tube 7.
5. Hemoglobin exited the column first because it was the larger of the two.