College of the Canyons: Introduction to Biotechnology

Gel Filtration: Post Lab Write Up

How are molecules separated using gel filtration?

By size primarily, larger particles emerge later (see diagram in lab)

Define filtration. How is gel filtration different than most familiar forms of filtration?

Sieving of particles under pressure. Most filtration uses a mesh to hold back larger particles (like making coffee). This type of filtration is in reverse in that larger particles are allowed to pass, and the smaller particles are held back.

Describe how separation occurs with the albumin, and salt solution present. Include the role of the column beads in this separation. Draw an example of a column bead during separation.

Albumin does not enter so it bypasses beads and emerges first. See diagram in lab manaul for clarification on the mechanism of separation.

List three ways you can test for the presence of a protein. Name technique/ technology, do not describe in gory detail.

Colorimeter: i.e. add reagent and if protein present will see color
Spectrophotometry: use spect. to detect protein (absorbs in the non visible light range.)
Reactions with antibodies (ELISA) ***others?***

5. When the lab was redone with the student being informed about the actual protein and salt concentrations, how did this effect the interpretation of the data? Cite 3 specific ideas regarding this.

With knowledge that protein total was 200 ug, the amount in the vials was likely reduced.
Negative controls revealed the light blue samples had no protein.
Salt concentrations shopuld be verified with controls as the meters are not very accurate.
Simply put, using postive and negative controls are very useful in helping one guage the amounts in samples as they amount in the fractions collected (vials 1-10) can not exceed the amount added!

What is meant by the term “desalting?” TO remove ions. This is common in protein purification as the salts are often cofactors for certain enzymes. These enzymes may degrade the protein (hence the name “protease”. Often DNA-ases, RNA ases are deactivated in a similar way (removing Mg++, Ca++ and other key ions).

7. Name 5 ways, and briefly describe how colorimetric data can be made more accurate by the person conducting the test. 1. Use a better scale with more numbers (200, 190, 180 ug/ml etc). 2. Collect positive and negtive controls for every test. 3. Collect more, smaller samples in each fraction.
4. Use transparent samples to compare to (like actual vials with control amounts of proteins.)
5. REPEAT the experiment.

8. Filtration is a mechanical method. Why would this be preferred method for purification of proteins as opposed to a chemical means of separation? Filtration is very gentle. Chemical methods can alter the tertiary and quantinary structure of proteins, thus effecting their functionality, marketability, etc.