Exp.5 Gel filtration Chromatography

Introduction：

Gel filtration Chromatography is used to separate large molecules on the basis of size. It is a widely used technique for the purification and analysis of synthetic and biological polymers, such as proteins, polysaccharides and nucleic acids etc. As the solution travels down the column some small particles enter into the pores of the beads, therefore move through the bed more slowly, while larger molecules enter less or not at all and thus move through the bed more quickly. Both molecular weight and structure contribute to the degree of retention.

Reagents:

1. egg protein and NaCl mixed solution
2. Ninhydrin reagent
3. Silver nitrate(0.6M $AgNO_3$)
4. Gel

Procedure：

1. Prepare the gel bead column (Equilibration):

Gently put cotton into the bottom of the column. Use the dropping pipette to transfer beads into the column. Be gentle. Do not allow gaps or bubbles to form. Catch the run-off with a small beaker.

2. Eluting and Cellecting:

Obtain 5 drops of sample solution and add it at the top of the column. The solution will enter the gel. As it does, continue to add 1ml of buffer solution (or H_2O) at the top till the sample is 2~3cm below the top. Add buffer (or H_2O) at the height of 5 cm above the top.

Collect the eluent, 10~20 drops per tube, beginning with the tube labeled "1."

3. Qualitative assay:

Each tube is separated into two parts, one is for chloride presence test and the other is for protein presence test.

4. Cleaning up and Recycling the gel:

Clean up the column as follows: Gently invert and shake the beads into the recycle beaker. Be patient. Some beads will come out the end. Then you can add some buffer solution (or H_2O) and shake. The rest of the beads will eventually come out.

a	Tube1~5	每管各收集10滴	
b	Tube6~8	每管各收集20滴	再從Tube6~8各分一半到Tube(6-1)~(8-1)，此六管各有等量的一半
c	Tube9~13	每管各收集10滴	

Results：

Record your results.

請用【+】或【-】紀錄於表上

For protein test: 【+】表示藍色至藍紫色； 【-】表示其他顏色

For Cl- test: 【+】表示有白色沉澱； 【-】表示無沉澱

	Gel filtration chromatography							
Ninhydrin (for protein test)	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8
$AgNO_3$ (for Cl^- test)	Tube 6-1	Tube 7-1	Tube 8-1	Tube 9	Tube 10	Tube 11	Tube 12	Tube 13

Discussions：