LS2102 : Gel Filtration Chromatography Assignment

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1 Question 1

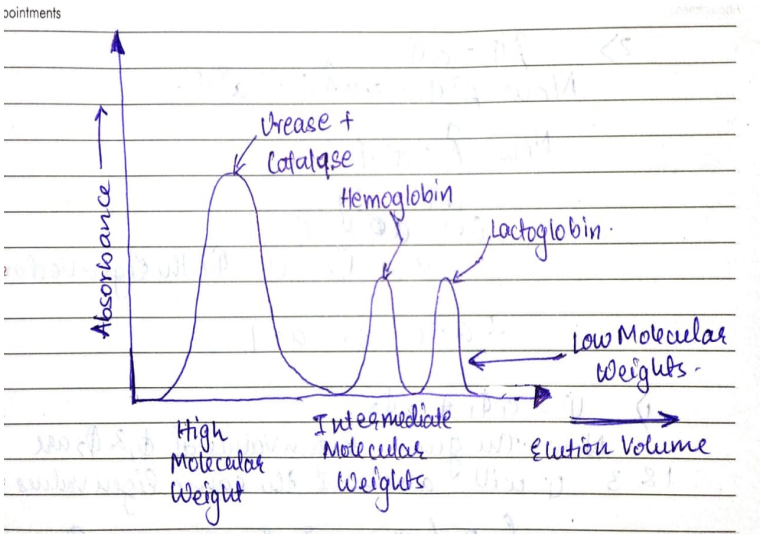
The exclusion range of the gel is given to be 120,000 Da - 25,000 Da. That means the molecules whose Molecular weight does not lie in that range are going to be eluted first and the small molecules in that range will be eluted later.

The Molecular weights for the proteins are given to be :

1. Urease = 482,700 Da
2. Catalase = 242,500 Da
3. Lactoglobin = 37,100 Da
4. Hemoglobin = 64,500 Da

Thus, we can see that the weights of Urease and Catalase are greater than the exclusion range thus they will be excluded from the beads and eluted first. The method of gel filtration chromatography is not great at purifying larger molecules because some small molecules might not enter the porous beads and might elute with the larger molecules. Thus we cannot get a 100% purifucation for Urease because there will always be smaller molecules.

However for lactoglobin and Hemoglobin it will be different both this molecules will be eluted later. As Hemoglobin is still heavier in size it will elute first from the gel matrix of the beads followed by lactoglobin. (However it depends on the flow rate of the column, If its too high you wont be able to separate Hemoglobin from lactoglobin.) Hence we will be able to purify lactoglobin from the protein mixture with this method.



2 Question 2

Assumption : We will assume that the protein given to us has a hydrophobic part on the chain.

Phosphorylation of an amino acid like threonin, serine, etc can cause the protein to be activated or cause some structural changes. When the serine(S) is mutated to Glutamic Acid(E) it mimics the same effects as the protein would experience if it was phosphorylated. These amino acid mutations to induce phosphorylation effects in the protein is called Phosphomimetics.

In the first case we can see that the wild type protein was applied to a column in the presence of Tris HCl(pH = 7.4) as buffer. We got a sharp peak for 145kDa. Now the mutation was made which probably caused a structural change in the protein. Causing it to break into two strands of approximately 72kDa each. Thus we get a huge peak at that value and a small one at 145 kDa. This small one might have been caused because not all the protein was mutated and retained their structures. Also few of those 72kDa subunits clumped together due to hydrophobic interaction and formed a protein with 145kDa peak.

In the second case the same mixture was applied in the column but this time with phoshphate buffer (pH = 7.2) as the mobile phase. Now we have assumed the chain to have a hydrophobic part. The phosphate buffer creates a phase suitable for hydrophobic interactions between these certain parts. Thus if the hydrophobic chains somehow interacted to form dimers or trimers we can infact observe a broad peak in 210 kDa region. We see a huge peak in 145 kDa this might be because the 72kDa proteins may have interacted to form a dimer of 145kDa. Similarly three 72kDa or 145kDa and a 72kDa might have formed a trimer or a dimer respectively. Thus we see a short peak in the 72kDa and a broad peak in the 210kDa.