Gel Filtration Chromatography

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August 19, 2023

1 Aim

Determination of Column Dead Volume using Gel Filtration Chromatography

2 Principle

The seperation between different molecules is done on the basis of molecule size and shape. This experiment is done in a cylindrical column with a stationary phase which are beads made up of pores that span the long column kept in a mobile phase or buffer. Smaller molecules spend a larger time inside the beads than the larger molecules and thus, the smaller molecules elute later(after a larger volume of the mobile phase has passed through).

3 Materials Used

1. Sephadex G-75 as the packing material
2. Blue Dextran as the dye to measure the column void volume
3. NaCl Buffer
4. Micropipettes
5. Spectrophotometer
6. 1.5 ml tubes

4 Procedure

1. The column was given to us filled with **Sephadex G-75** as the stationary phase material and NaCl as the buffer solution which was our mobile phase
2. Around 2% of the bed volume, Blue dextran was measured, added and mixed with the buffer.

3. The preparation was then poured into the column.
4. And then buffer was added continuously to put pressure on the Blue Dextran Molecules.
5. The buffer was also added as so as to prevent the column from running dry.
6. The buffer was drained out onto another tube and was done so till the Blue Dextran Started pouring out.
7. Then on the first appearance of the Blue Dextran molecules, they were poured onto 1.5 ml tubes.
8. Then the tubes were filled with 500 μl buffer.
9. The stopcock was closed after all the Blue Dextran had passe through it.
10. The absorbance of every sample was recorded at 610 wavelength. The sample with the maximum absorbance was noted.
11. The volume of the buffer collected in the 15mL tube was later added to the volume of the Blue Dextran collected till the maximum absorbance.The total is the dead volume V_0 of the tube.

5 Results

Sample	Absorbance Value
1	0.630
2	1.076
3	0.991
4	0.694
5	0.181

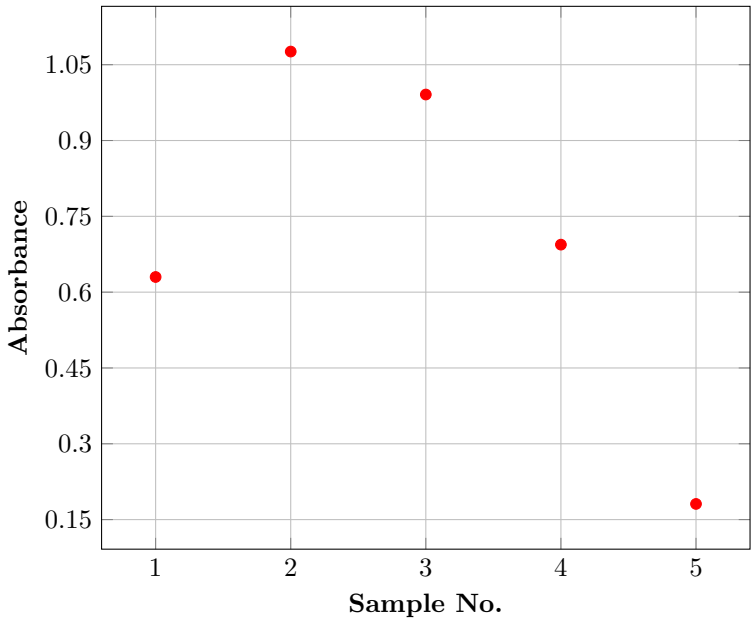

Figure 1: Absorbance of each sample

5.1 Column Data

- Bed Volume = 22.4 ml
- Amount of Blue Dextran Added over the volume = 448 μL
- Amount of buffer collected in the 15mL tube = 7mL
- Amount of Blue Dextran till the maximum absorbance = 1 mL

6 Conclusion

We conclude that the total void volume for the column and stationary phase is around 8mL(7mL+1mL).