**Lab sheet #5**

**-Gel Filtration Chromatography-**

**-Objectives:**

* Separating mixture of blue dextran and bromophenol blue based on their molecular weights by gel filtration chromatography.

**-Method:**

**You will separate a mixture of blue dextran [sugar] with m.wt.= 2,000,000 Da and bromophenol blue [dye] m.wt.= 669.99 Da using Sephadex G-100 (4,000 -150,000 Da).**

1. Carefully remove the layer of 0.5M buffer solution from above the resin bed using a pasture pipette, leaving only a very thin layer of solution. *Do not expose the gel bed.*
2. Using the pasture pipette, very carefully load 1 ml of the sample mixture solution on the top of the resin, by allowing the sample solution to slide on the wall of the column. *Be careful not to disturb the gel beads.*
3. Open the screw clip and start to collect fractions of about 3 ml each.
4. Allow the sample mixture to enter the gel bed before you start adding the 0.5M buffer solution. Continue collecting 3 ml fractions.
5. Start adding the 0.5M buffer solution carefully, and continue collecting 3ml fractions, collect until the samples completely eluted. Keep the top of the resin covered with 0.5M buffer all the time.
6. Read the absorbance of each fraction at 560 nm by using the spectrophotometer against a blank of 0.5M buffer and record it in the result table.

**-Results:**

* Record the absorbance in the table. Then plot the absorbance at 560 nm against fraction number.
* Identify the peaks and determine the elution volume (Ve) of blue dextran and bromophenol blue from the chromatogram.
* Calculate the void volume (V0).

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| **Fraction number** | **Absorbance at 560 nm** |
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**Related questions:**

1. What is the relation between the Ve of a molecule and its molecular weight?

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1. Define the elution volume (Ve).

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1. Which substance will elute first? Why?

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1. The separation in this chromatography method based on what?

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