**BCH 333** Gel filtration chromatography

**Lab Sheet #5**

You are provided with the following :

-A gel filtration column packed with Sephadex G-100 in 0.5M phosphate buffer pH7.4.

 -Sample mixture, of blue dextran [sugar] with m.wt.= 2,000,000 Da and bromophenol blue[dye] m.wt.= 669.99 Da .

**Materials:**

**-Chemicals:** ……………………………………………………………………………………………………………………………………………………………………………………………………

……………………………………………………………………………………………………………………………………………………………………………………………………

**-** **Glassware:**

……………………………………………………………………………………………………………………………………………………………………………………………………

**-Instruments:**

……………………………………………………………………………………………………………………………………………………………………………………………………

**Method:**

**Procedure:**

**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 salt solution. Do not expose the gel bed.

**2.** Using the pasture pipette, very carefully load the sample mixture solution on the top of the resin, by allowing the sample solution to slide on the wall of the column. Care should be taken 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.

**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 560nm by using the spectrophotometer against a blank of 0.5M buffer, and record it in the result table

**Results:**

|  |  |
| --- | --- |
| **Fraction number** | **Absorbance at 560 nm** |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |

- Plot a graph of absorbance at 560 nm against fraction number.

-Calculate:

1- The void volume:

……………………………………………………………………………………………………………………………………………………………………………………………………

2- The elution volume for each molecule:

……………………………………………………………………………………………………………………………………………………………………………………………………

……………………………………………………………………………………………………………………………………………………………………………………………………