**BCH 333** Cation exchange chromatography

**Lab Sheet #6**

You are provided with the following :

- A Cation exchange chromatography column Sephadex C-25,

The sample is a mixture of two proteins, myoglobin and cytochrom C, [pI=7.2 and 10.2 respectively] dissolved in phosphate buffer[pH=8]. Separate the mixture by using the buffer[pH=8 and 1M NaCl.

**Materials:**

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

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**-** **Glassware:**

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**-Instruments:**

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**Method:**

**Procedure:**

**1-**Carefully remove the layer of phosphate buffer from above the resin bed using a pasture pipette, leaving only a very thin layer of buffer. Do not expose the gel bed .

**2-** Very carefully load the sample mixture solution [using the pasture pipette] 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 phosphate buffer pH 8.0 .Continue collecting 3ml fractions .

**5-**Collect the fraction of the first protein , and make sure that it is completely eluted.

**6-** Then start adding the 1 M NaCl solution , do not forget to mark the fraction where you started adding the 1M NaCl solution. Continue collecting 3ml fractions until you ensure that the second protein has been completely eluted .

**7-** Read the absorbance of each fraction at 410nm by using the spectrophotometer, using the phosphate buffer as a blank for the fractions that were eluted using the buffer , and use 1 M NaCl as a blank for the fractions that were eluted by using the salt.

**8-** Record the absorbance of each fraction in the result table .

**Results:**

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| **Fraction number** | **Absorbance at 410 nm** |
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- Plot a graph of absorbance at 410 nm against fraction number.

-Identify each peak.