**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:** ……………………………………………………………………………………………………………………………………………………………………………………………………

**-** **Glassware:**

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**-Instruments:**

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**Method:**

**Procedure:**

**-**You are provided with Cation exchange chromatography column, separate the sample given to you. If you know, that 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 phosphate buffer[pH=8] and 1M NaCl.

**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. Then 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.